CoCo Administration and User Manual

Introduction

The ChIP-on-Chip online (CoCo) application helps you analyzing your ChIP-on-chip results. CoCo integrates results from ChIP-on-chip experiments together with in situ and gene expression profiling results. All these datasets are put in a genomic context and displayed as meaningful colored pictures. User can browse results in the fashion of a genome browser and define regulatory regions together with target genes while browsing.

User Manual

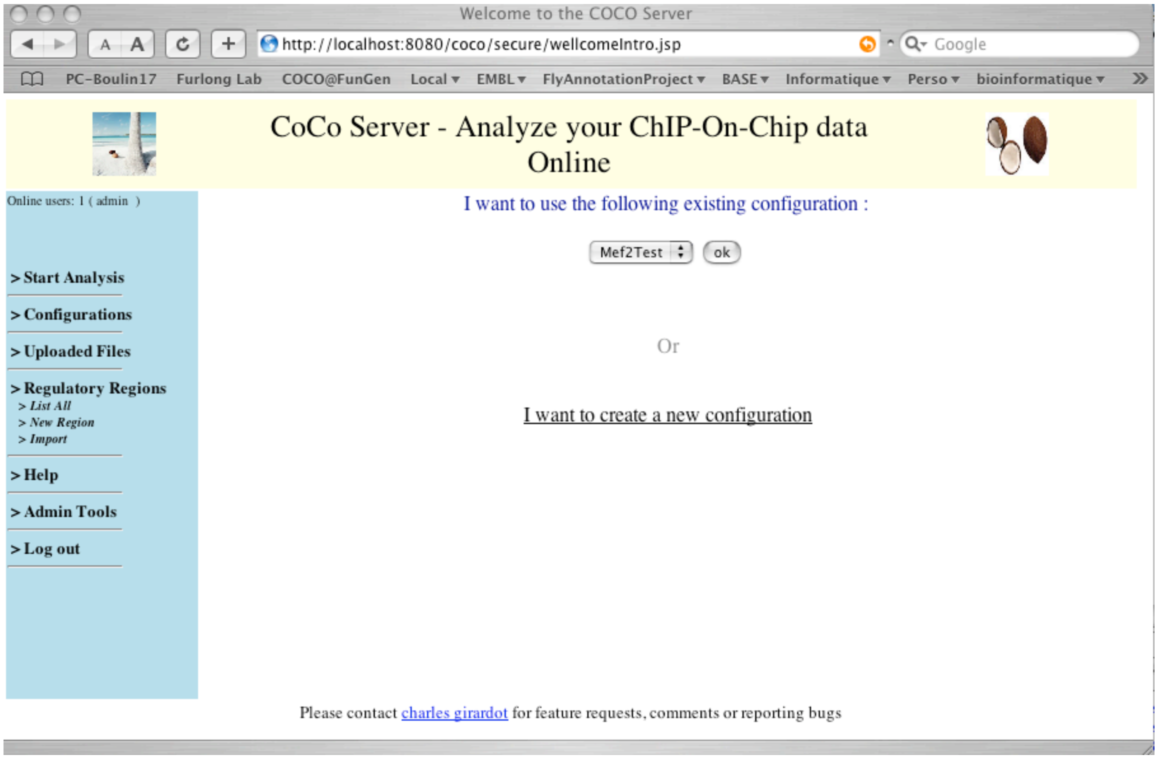
Introduction

CoCo is a web application that allows the user to search, visualize and store different data associated with gene expression. The program integrates ChIP-on-chip, expression profiling and in-situ hybridization data to create a user specified 'configuration'. The data can then be visualized and searched on a user-friendly interface, which displays all data as well as the surrounding genes. The user can zoom in and out to different genomic regions and save images of the displayed data.

1. Login in – menu overview

First ask your CoCo administrator for a login. If you are the CoCo admin, then please consult the *installation and maintenance section.*

You can then point your browser to http://<servername>/coco e.g. http://localhost/coco and login.
Once login, you'll be looking at page like the below picture.



CoCo Server - Analyze your ChIP-On-Chip data Online

I want to use the following existing configuration :

Or

I want to create a new configuration

Please contact charles girardot for feature requests, comments or reporting bugs

1.1 Menu Description

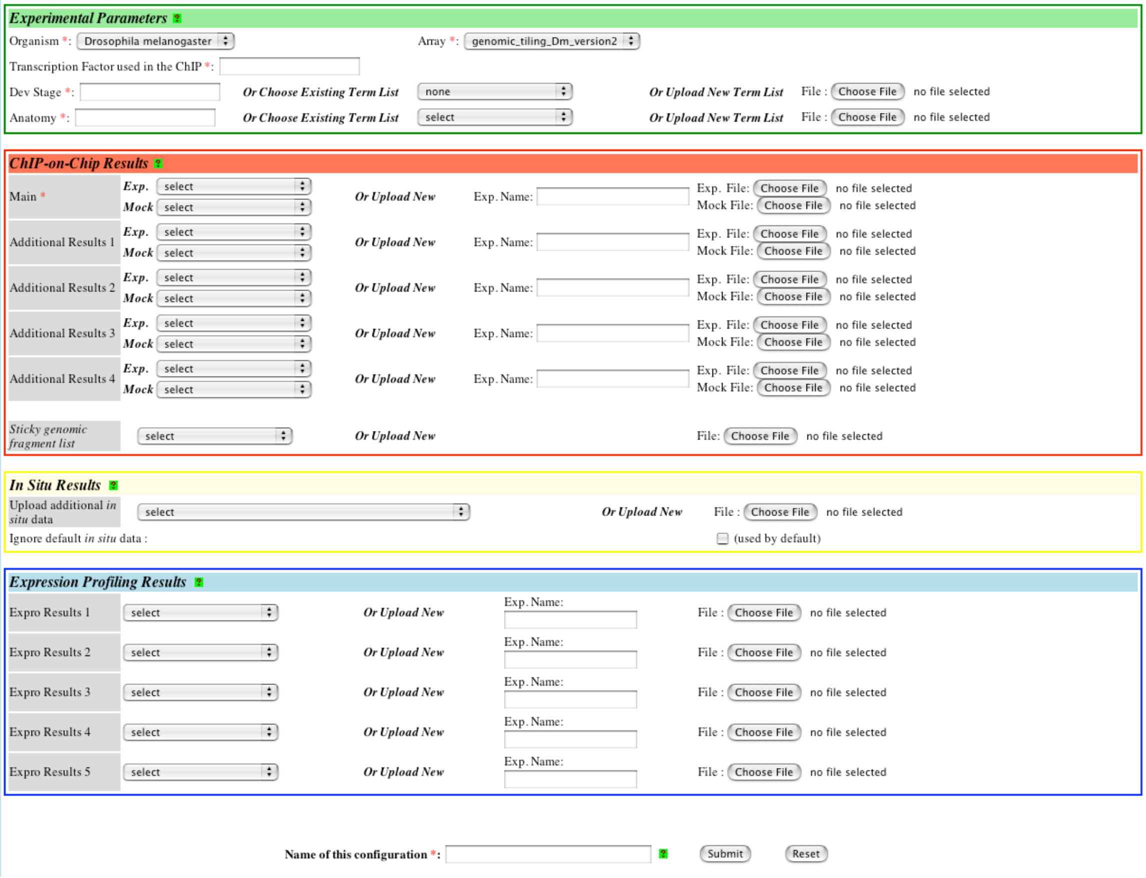
The left menu is always available. Depending on whether you have started an analysis or not, different sub-menus are made available.
Main menu items are:

- **Start Analysis**: the first page you see when you log in. This page allows you to either select an existing configuration to work with or create a new one
- **Configurations**: this menu lets you view and manage your configuration(s). Once a configuration has been selected, sub-menus will appear.
- **Uploaded Files**: this menu lets you view and manage your files(s). This is where you should go to delete or share your files.
- **Regulatory Regions**: this menu lets you view, manage and import regulatory regions.
- **Help**: provide some help like a link to download this document
- **Admin Tools**: (available only if user has the role 'admin') Allow to reload properties after a change e.g. after addition of a new chip or db connection settings update
- **Log out**: close work session

In addition to these basic operations, other context-dependent possibilities will be offered.

2. Creating a Configuration

A *configuration* is a space where users can integrate ChIP-on-chip datasets with microarray expression profiling data, in situ patterns and genome annotations. In addition to the organism and the ChIP-on-chip microarray design, a configuration is given a developmental stage term list and an anatomy term list. Once a configuration has been created, users can visualize all data on interactive pictures that can be accessed in a genomic browser fashion. Input data format is tab-delimited and data should be already normalized. Please see the *file format* chapter to know more about file formats.



Experimental Parameters

Organism *: Drosophila melanogaster — Array *: genomic_tiling_Dm_version2

Transcription Factor used in the ChIP *:

Dev Stage *: — *Or Choose Existing Term List* none — *Or Upload New Term List* File : Choose File no file selected

Anatomy *: — *Or Choose Existing Term List* select — *Or Upload New Term List* File : Choose File no file selected

ChIP-on-Chip Results

Main *	***Exp.*** select ***Mock*** select	***Or Upload New***	Exp. Name:	Exp. File: Choose File no file selected Mock File: Choose File no file selected
Additional Results 1	***Exp.*** select ***Mock*** select	***Or Upload New***	Exp. Name:	Exp. File: Choose File no file selected Mock File: Choose File no file selected
Additional Results 2	***Exp.*** select ***Mock*** select	***Or Upload New***	Exp. Name:	Exp. File: Choose File no file selected Mock File: Choose File no file selected
Additional Results 3	***Exp.*** select ***Mock*** select	***Or Upload New***	Exp. Name:	Exp. File: Choose File no file selected Mock File: Choose File no file selected
Additional Results 4	***Exp.*** select ***Mock*** select	***Or Upload New***	Exp. Name:	Exp. File: Choose File no file selected Mock File: Choose File no file selected
Sticky genomic fragment list	select	***Or Upload New***		File: Choose File no file selected

In Situ Results

Upload additional *in situ* data — select — ***Or Upload New*** File : Choose File no file selected

Ignore default *in situ* data : □ (used by default)

Expression Profiling Results

Expro Results 1	select	***Or Upload New***	Exp. Name:	File : Choose File no file selected
Expro Results 2	select	***Or Upload New***	Exp. Name:	File : Choose File no file selected
Expro Results 3	select	***Or Upload New***	Exp. Name:	File : Choose File no file selected
Expro Results 4	select	***Or Upload New***	Exp. Name:	File : Choose File no file selected
Expro Results 5	select	***Or Upload New***	Exp. Name:	File : Choose File no file selected

Name of this configuration *:

The Configuration form in details

This form has four main sections:

1. Experimental Parameters
2. ChIP-on-chip Results
3. In Situ Results
4. Expression profiling Results

Fill in the four section, give your configuration a meaningful name and click “Submit”. CoCo will then parse and validate all your files. It will convert ids found in Expro and In Situ files to internal ids, and finally pass all these data to R (format communication is GFF) which will then index them. This process is time-consuming, so please be patient, it should last for a couple of minutes.

Remark: the form usually offers to either select a file stored on the server or upload a new one. Once you uploaded a file, it becomes available to you and other users if you decide to share it. To keep interface happy, thing about:

- DO NOT HAVE SPACES OR characters like !@#$%^&*{}<>?/\|~`
- Using meaningful file names as file names are displayed in selection box
- Using *short* file names as file names are displayed in selection box. Try to not exceed 20 characters.

- Deleting unused old files
- It might happen that an error occur while loading a configuration (mistake in files…) before loading completes. In such situations, CoCo tries to delete uploaded files but it sometimes happen that CoCo can't do it. After a failure, always check if your files have been saved on the server or not (using the "Uploaded Files" menu) and delete those that CoCo haven't succeeded to clean as they are certainly corrupted. If you leave them, you won't be able to upload a file having the same name and you will pollute the server with corrupted files that will appear in select menus.

Note that mandatory fields have a red star close to their name.

2.1 Experimental Parameters Section

Select the organism and the microarray design used to perform the ChIP-on-chip experiments

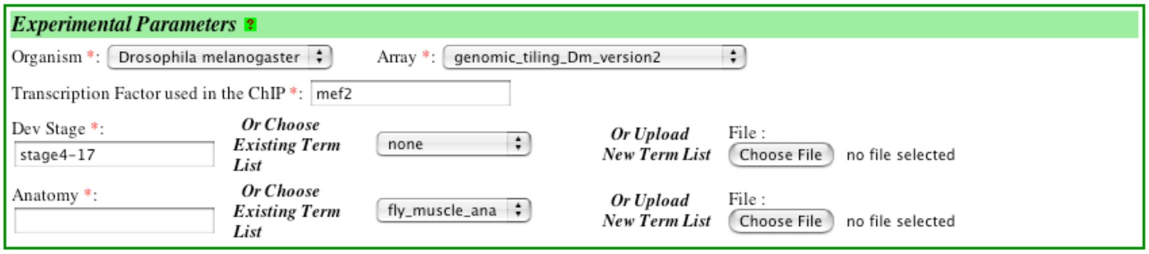
Select the transcription factor used in the Chromatin IP. In case you plan to mix datasets gained with ChIP using different transcription factors, you should provide the one used for the *main* ChIP-chip experiment. We'll come back to this later.

Indicate the Developmental Stage and Anatomical term lists the configuration should focus on. These term lists are supposed to reflect your experimental setup and will be used to color genes using available *in situ* data. For example, if you used samples collected from the heart muscle at developmental stage 2, you are certainly interested to clearly see those genes expressed in the heart at the same stage or maybe at stages 1 to 4. CoCo offers you different ways to provide this term lists but you should use <u>only one</u> of them:

1. A free text box: this option is relevant when you have a unique term e.g. "heart muscle". For the developmental stage only, you have the option to give a range. This must be in the form <prefix><from>-<to> e.g. "stage1-5" would translate in term list [stage1, stage2, stage3, stage4, stage5]. Note that <prefix> can't have spaces and <from>/<to> part must be numbers. If you break these rules, form validation fails.
2. Select an existing term list: here you can select term lists already available on the server. A term list becomes available as soon as you uploaded such a file using the third option.
3. Upload a file containing terms. The file should have one column, one term per line.

Now comes the question: what terms should I use? Well… this depends what you used in your in situ files: you must use the same and this is case-sensitive! Dealing with in situ data is quiet complex and can't yet be generalized even though ontologies for both developmental stages and anatomy exist for several organisms. The basic problem is that in situ results have usually not been annotated using those. The other problems is that these ontologies have thousands of terms with complex relations. Because of this, you always have to pre-process in situ results and turn annotations into simpler classifications. We never succeeded to go around this pre-processing step so we decided to have a simpler approach in CoCo that allow to cope with all situations : as you pre-process in situ results, you should build relevant term lists. Once done, give them a meaningful name and upload them in CoCo as admin…share them with relevant groups and users will simply have to select the one they need.

On the example picture below, we create a configuration for a ChIP-chip time series of the ***Drosophila melanogaster*** transcription factor ***mef2***. The microarray used for the ChIP-chip experiments is ***genomic_tiling_Dm_version2***. The spatio-temporal focus is set to ***stage4*** to ***stage17*** and we provide a anatomical term list ***fly_muscle_anatomical_terms.txt*** by selecting a Term List File already present on the server.
Note the "stage4-17" short cut used to specify a range. When "Dev Stage" field is filled, CoCo tries to guess if you mean a single developmental stage or a range by analyzing your input. If your input look like <word><number1>-<number2>, CoCo understands that you meant "all combinations of <word><number> where <number> increments from <number1> to <number2>". For "stage 4-17", it translates into the list "stage4, stage5, …, stage16, stage17". This feature is only available for the "Dev Stage" field and no spaces can be used i.e. "embryonic stage1-17" won't be understand as a stage by CoCo. In such a situation, simply create a file listing all your terms (i.e. embryonic stage1, embryonic stage2, …, embryonic stage17), one term per line.



Experimental Parameters ?

Organism *: Drosophila melanogaster

Array *: genomic_tiling_Dm_version2

Transcription Factor used in the ChIP *: mef2

Dev Stage *: stage4-17 — *Or Choose Existing Term List* none — *Or Upload New Term List* File : Choose File no file selected

Anatomy *: — *Or Choose Existing Term List* fly_muscle_ana — *Or Upload New Term List* File : Choose File no file selected

2.2 ChIP-on-chip Results Section

CoCo accepts up to five ChIP-chip experiments. One experiment is mandatory and is call the *main* experiment. When uploading more than one experiment, try to define the most relevant as the *main* experiment: CoCo uses the *main* experiment data in different situations where it is not possible to mix all datasets together (or not yet implemented!). As you read this document, we'll point you such situations.

For each ChIP-chip experiment, you should either:

- select files from drop down menus or,
- upload new file(s) and give these file(s) an experiment name. Please use short experiment name (e.g. 10 letters)

To cope with common design, ChIP-chip experiments can be made of two different files i.e. two different result sets: a test and a mock result set where the mock represents results obtained in the same conditions as test but using a mock antibody in the ChIP step. In such designs, two hybridizations (using two channels platforms) are performed: the first hybridization measures test sample signal over genomic DNA while the second measures the mock signal over genomic DNA.

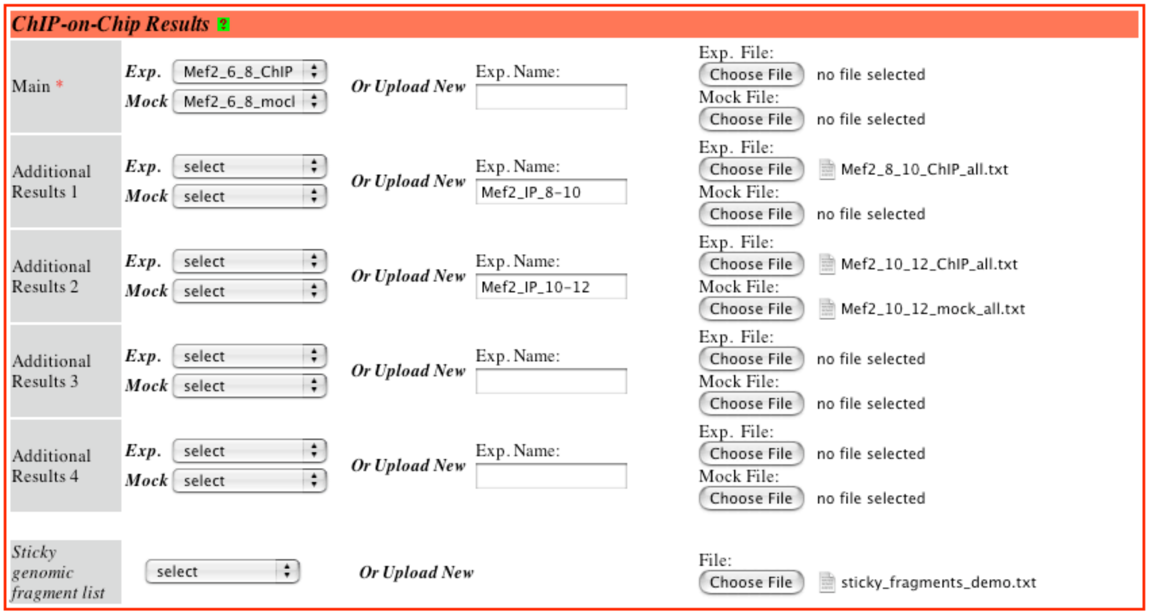
Providing mock results is optional.

Finally, at the end of the form section, you can provide a "sticky" file. This file is a single column file listing microarray features (one per line) found to be "sticky". Features marked as "sticky" will be masked on pictures. We initially introduced this "sticky" notions based on two different observations: (1) some array features always show up as enriched and (2) some features are not clearly mapped to the genome (especially after a new genome assembly has been made available). In both situations, we want to flag them clearly and ignore them in result list. After using CoCo, we actually found that this "sticky" file shows to be useful in other situations e.g. you want to mask lots of features to easily trace the behavior of very interesting regions over different datasets.

<u>Important:</u> The order in which you specify datasets is kept in CoCo and in displays.

The picture below shows an example where we mixed experiments already uploaded on the server with new experiments (new file upload). We also upload a "sticky" files.
Note that:

- you must not give an experiment name for experiments that are already on the server
- you must give an experiment name for new experiments
- each track might take a "mock" dataset or not



ChIP-on-Chip Results ?

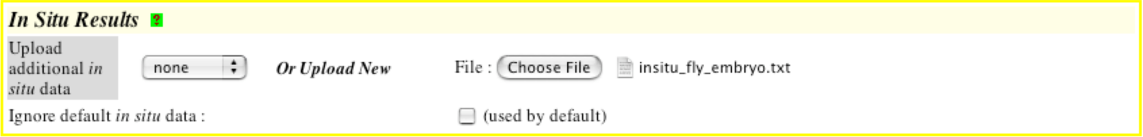
Main *	***Exp.*** Mef2_6_8_ChIP ***Mock*** Mef2_6_8_mocl	***Or Upload New***	Exp. Name:	Exp. File: Choose File no file selected Mock File: Choose File no file selected
Additional Results 1	***Exp.*** select ***Mock*** select	***Or Upload New***	Exp. Name: Mef2_IP_8-10	Exp. File: Choose File Mef2_8_10_ChIP_all.txt Mock File: Choose File no file selected
Additional Results 2	***Exp.*** select ***Mock*** select	***Or Upload New***	Exp. Name: Mef2_IP_10-12	Exp. File: Choose File Mef2_10_12_ChIP_all.txt Mock File: Choose File Mef2_10_12_mock_all.txt
Additional Results 3	***Exp.*** select ***Mock*** select	***Or Upload New***	Exp. Name:	Exp. File: Choose File no file selected Mock File: Choose File no file selected
Additional Results 4	***Exp.*** select ***Mock*** select	***Or Upload New***	Exp. Name:	Exp. File: Choose File no file selected Mock File: Choose File no file selected
Sticky genomic fragment list	select	***Or Upload New***		File: Choose File sticky_fragments_demo.txt

2.3 In situ Results Section

The in situ result section lets you attach in situ results to your configuration. Because preparing in situ results might be quite time-consuming and delicate task, CoCo offers the possibility to define a default in situ dataset per organism. This has to be configures by the administrator and will be available to all users. If present, this dataset is included by default in every configuration but you can indicate that you don't want to use it.

In addition, you can specify or select a file holding additional in situ results (e.g. collected in your lab). Note that the file name will be used as the "dataset" name in displays so you might want to keep it **short and meaningful**.

The picture below shows an example where we upload in situ results in addition to use the default in situ file (in this case BDGP in situs).



In Situ Results ?

Upload additional *in situ* data: none — *Or Upload New* — File : Choose File insitu_fly_embryo.txt

Ignore default *in situ* data : □ (used by default)

2.4 Expression Profiling Results Section

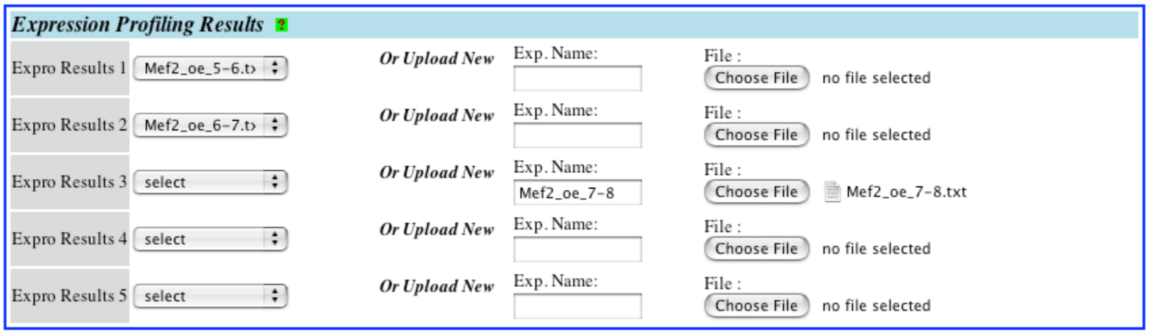
The last section lets you attach gene expression profiling results to your configuration. This section looks pretty much like the other sections. You can specify from 0 to 5 expression profiling datasets by either selecting available datasets or uploading new files. If you choose to upload new file(s), you must give an experiment name to each of them and you should, as usual, keep these names **short and meaningful**.

Important: The order in which you specify datasets is kept in CoCo and in displays.

The picture below shows an example where we mixed experiments already uploaded on the server with new experiments (new file upload).

Note that:

- you must not give an experiment name for experiments that are already on the server
- you must give an experiment name for new experiments



Expression Profiling Results ?

Expro Results 1	Mef2_oe_5-6.t›	*Or Upload New*	Exp. Name:	File : Choose File no file selected
Expro Results 2	Mef2_oe_6-7.t›	*Or Upload New*	Exp. Name:	File : Choose File no file selected
Expro Results 3	select	*Or Upload New*	Exp. Name: Mef2_oe_7-8	File : Choose File Mef2_oe_7-8.txt
Expro Results 4	select	*Or Upload New*	Exp. Name:	File : Choose File no file selected
Expro Results 5	select	*Or Upload New*	Exp. Name:	File : Choose File no file selected

2.5 Naming examples

As you might have noticed, we quite insist on names you should give to your files, experiments... This is because these are used in displays and long names will badly display especially in the genome browser. Please try to keep experiment names less than 15 letters (best is 10).

3. Browsing data

Once you created a configuration, you can start working. There are two main visualization pages:

1. The Overview page
2. The Genome Browser

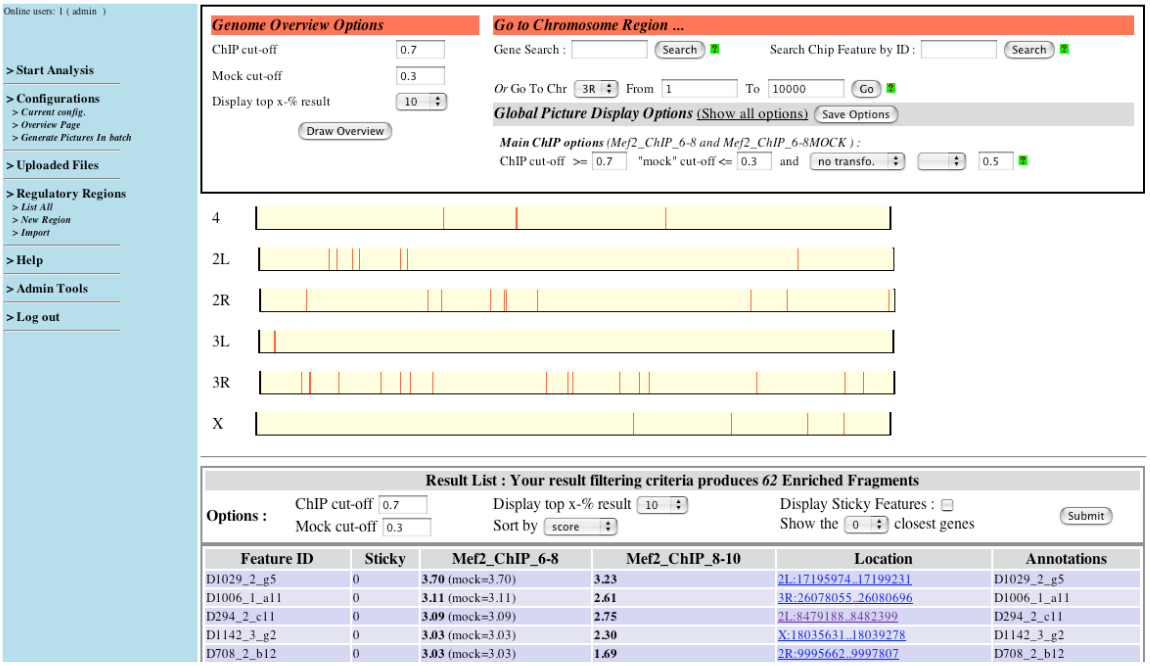
3.1 Overview Page

The overview page (see picture below) depicts each chromosome and a result table summarizes all ChIP-on-chip results. Note that the table keeps the order in which the ChIP-chip datasets have been submitted. Thanks to chromosome pictures, users can gain understanding about how enriched regions spread across the genome and thus identify clusters of enriched regions.

At the top left of the page, in "***Genome Overview options***", you can set cut-offs (used to define enriched regions in the chromosome pictures) and compute pictures again.

At the bottom of the page, a result summary ("***Result List***") is presented in a result table. Here again, we can modify cut-offs are re-order the table (you can order by score or genomic location). You can also ask to display to *n* closest genes in an extra column. This feature might take some time and we recommend using it only for a subset of the results (this is controlled but the "*Display top x-% result*" option). If you choose to display sticky features, they will appear on a grey background.

The chromosome overview pictures are built with results of the main ChIP-chip experiment only. The same is true for the result table ordering (when ordering by score is selected). These are examples where it is a bit tricky to find a way to apply user selection to all results together…



Genome Overview Options

ChIP cut-off 0.7

Mock cut-off 0.3

Display top x-% result 10

Go to Chromosome Region ...

Gene Search :

Search Chip Feature by ID :

Or Go To Chr 3R From 1 To 10000

Global Picture Display Options (Show all options)

Main ChIP options *(Mef2_ChIP_6-8 and Mef2_ChIP_6-8MOCK) :*

ChIP cut-off >= 0.7 "mock" cut-off <= 0.3 and no transfo. 0.5

Result List : Your result filtering criteria produces *62* Enriched Fragments

Options : ChIP cut-off 0.7 Mock cut-off 0.3 Display top x-% result 10 Sort by score Display Sticky Features : □ Show the 0 closest genes

Feature ID	Sticky	Mef2_ChIP_6-8	Mef2_ChIP_8-10	Location	Annotations
D1029_2_g5	0	**3.70** (mock=3.70)	**3.23**	2L:17195974..17199231	D1029_2_g5
D1006_1_a11	0	**3.11** (mock=3.11)	**2.61**	3R:26078055..26080696	D1006_1_a11
D294_2_c11	0	**3.09** (mock=3.09)	**2.75**	2L:8479188..8482399	D294_2_c11
D1142_3_g2	0	**3.03** (mock=3.03)	**2.30**	X:18035631..18039278	D1142_3_g2
D708_2_b12	0	**3.03** (mock=3.03)	**1.69**	2R:9995662..9997807	D708_2_b12

From the overview page, several options are provided to switch in genome browsing mode:

1. you can click on chromosome pictures, this will open the genome browser centered on the region you clicked. The region displayed will be quite broad.
2. you can follow a link from the result table. The region displayed will be sharper, the size of it is actually a property the administrator can set. By default, it is 30 Kb.
3. you can use one of the search option: search by gene (use symbol or synonyms), search by microarray feature ID (the microarray is the tiling array, not expression microarray!) or specify a genomic location

Finally, you can set directly in this page the parameters you want to use in the genome browser mode (in "***Global Picture Display Options***"). This "***Global Picture Display Options***" panel as well as the search toolbox will be available in genome browser mode as well.

3.2 Genome Browser

From the overview page, users can start browsing data in a genome browser fashion. To enter the genome browser, users can search by gene symbol or chip feature ID, specify a genomic location, follow links provided in the result table or click on chromosome overview pictures. The genome browser is the place where all data are displayed together and certainly one of the main features of CoCo. As the user browses (or zoom in/out), CoCo assembles *genomic region views* representing the requested chromosomal region.

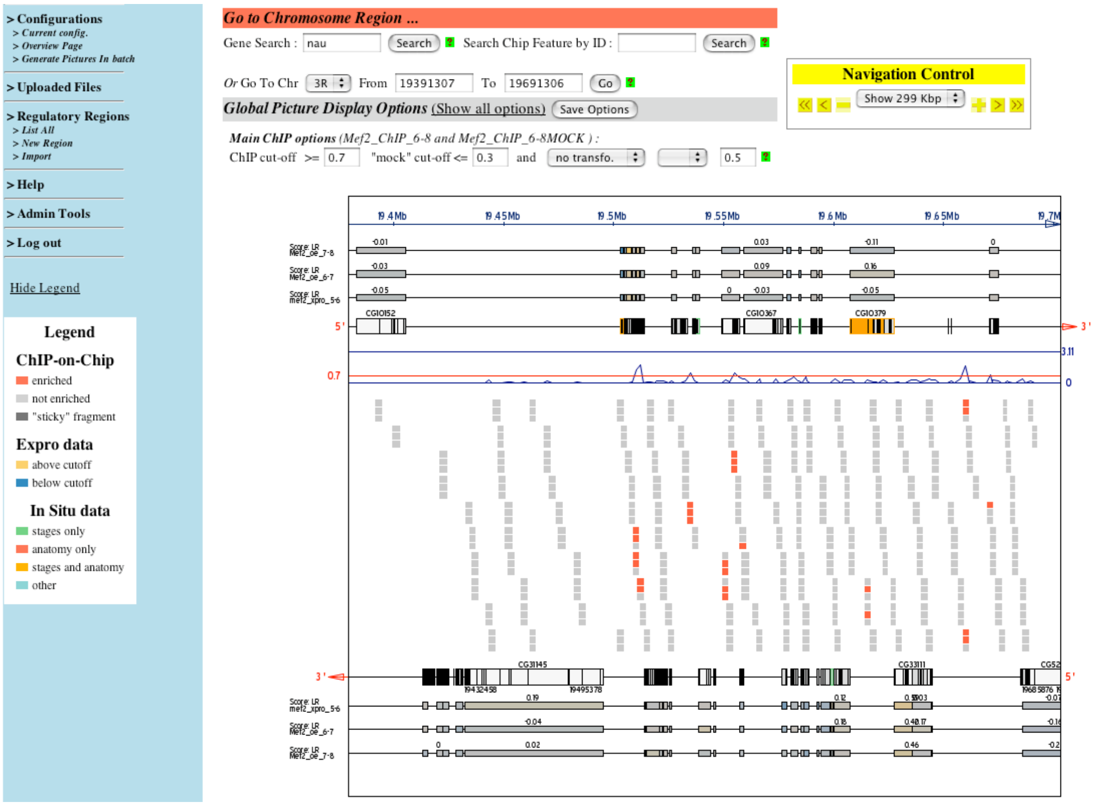
Down the "Navigation Control" panel, clicking the "View All Regulatory Regions Overlapping With…" will open up a new window displaying all regions found in the picture

3.2.1 Picture Description

Genomic region view pictures are organized in three main zones (see picture below). In the central part (ChIP-on-chip zone), each tiling array feature is represented as a rectangle colored in red or grey depending on whether its enrichment value is above or under the user-defined threshold (thresholds can be set for each dataset individually). A third color, black, is used for features defined as sticky. A rectangle is draw for each dataset microarray features resulting in the stacks as shown on the picture. To cope with time series, datasets order within stacks follows order given at configuration creation. The plus and minus genomic strands together with genes (including exon/intron boundaries) are represented above and below the ChIP-on-chip zone, respectively. Genes are colored according to available in situ patterns and four colors are used to reflect whether genes are expressed at the stages and/or anatomy specified in the configuration. Finally, the upper and lower zones represent expression values for genes found on the plus and minus strand, respectively. Each expression dataset has its own track and color-coded rectangles, aligned with their corresponding genes, are draw whenever result is available (in case more than one results is available for a gene, the mean together with standard deviation is used and displayed). Rectangles are colored using a color ramp from blue (under-expressed) to yellow (over-expressed) where the minimum and maximum values are user-defined.

Information about genes, expression values and enrichment folds is displayed while moving the mouse over the picture. In addition, clicking on genes or ChIP-on-chip features opens dialog pages allowing users to undertake actions like accessing gene or feature report page, creating regulatory regions or assigning genes to regions.

The picture below presents a genomic region view example where enriched features are found in a gene dense region. Here the use of CoCo is certainly needed to find which gene(s) are under control of Mef2.



Gene Search : nau

Search Chip Feature by ID :

Or Go To Chr 3R From 19391307 To 19691306

Show 299 Kbp

***Main ChIP options** (Mef2_ChIP_6-8 and Mef2_ChIP_6-8MOCK) :*

ChIP cut-off >= 0.7 "mock" cut-off <= 0.3 and no transfo. 0.5

3.2.2 Positioning picture parameters

Cut-offs used for color-coding are controlled in the ***Global Picture Display Options*** panel. Here you can position cut-offs for ChIP-chip and expro experiments. Each ChIP-chip experiment has its own settings while expression profiling cut-offs apply to all expression profiling experiments:

ChIP-chip parameters:

- if you did not provide mock results, a unique cut-off “ChIP cut-off” is available and features having a value greater or equal to this cut-off will be colored in red
- if you did provide mock results, additional cut-off and conditions can be set:
  - the mock cut-off must be set and is used to remove enriched features from the result set when their associated mock is greater or equal to this mock cut-off
  - you can define an additional condition that must be reached to define a feature as enriched: this condition uses either the ratio or the difference (as you set it) and a cut-off.

Three Expression profiling parameters must be set (if expression profiling datasets are included in the configuration):

- Minimun and Maximum values: between min and max, the color used will follow a color ramp from blue (min value) to yellow (max value). Below the min value and above the max value, color won't change anymore and remain yellow or blue.
- A "cut-off": this name might be badly chosen as this "cut-off" represents the center of your min/max couple i.e. the point where the color will be grey indicating that the gene is neither up- or down-regulated in the experiment. If the values found in your expression datasets are log ratios, this cut-off must be set to 0.

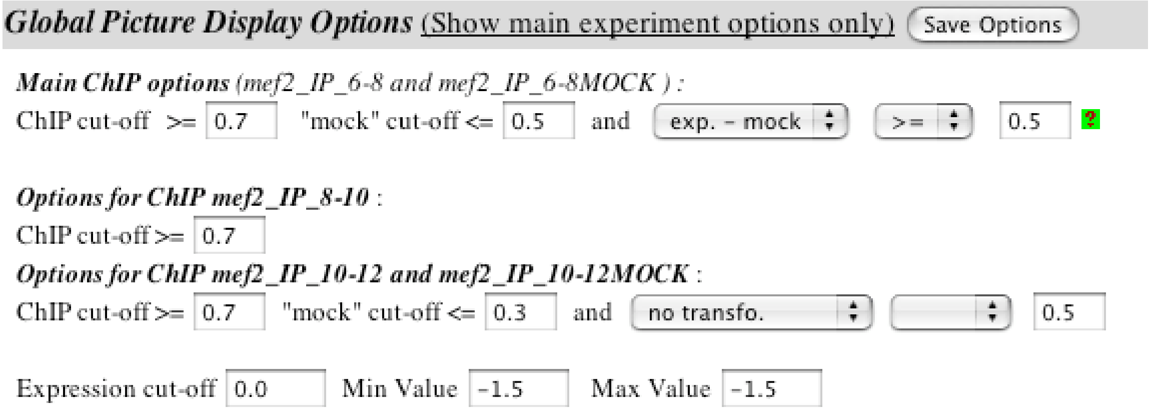
Once positioned, click "Save" to store them in the database so that they remain after you log out. This allows CoCo to load them the next time you log in. Using the "*Show all options*" / "*Show main experiment only options*", you can show or hide (partially) this panel.

In the example below, we have three ChIP-chip experiments, two of them have associated mock results. In the "main" experiment, we define enriched features as features where:

- the "ChIP" (understand enrichment of the test dataset) enrichment (here we loaded log transformed enrichment over genomic DNA) is greater them 0.7
- AND the enrichment in the mock dataset is less than 0.5
- AND the difference between test and mock value is more than 0.5

The second experiment has no mock data and thus a unique "ChIP cut off" can be positioned. Finally the third experiment, has no "transformation" condition i.e. enriched features have to be over 0.7 and less than 0.3 in the mock.

The configuration has expression profiling datasets which values are log-ratios. We then set expro cut-off to 0 and min/max to –1.5 / 1.5 respectively.



Global Picture Display Options (Show main experiment options only) Save Options

Main ChIP options (mef2_IP_6-8 and mef2_IP_6-8MOCK) :

ChIP cut-off >= 0.7 "mock" cut-off <= 0.5 and exp. - mock >= 0.5 ?

Options for ChIP mef2_IP_8-10 :

ChIP cut-off >= 0.7

Options for ChIP mef2_IP_10-12 and mef2_IP_10-12MOCK :

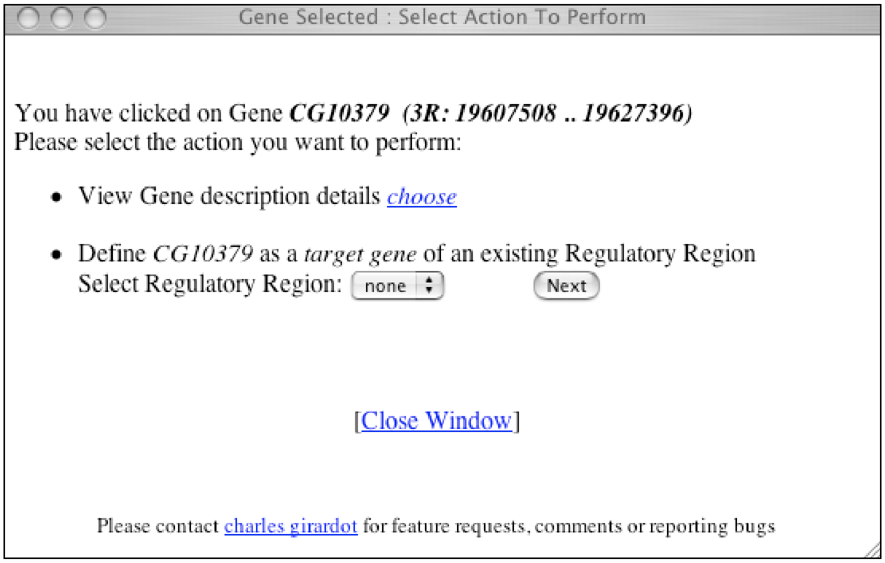
ChIP cut-off >= 0.7 "mock" cut-off <= 0.3 and no transfo. 0.5

Expression cut-off 0.0 Min Value -1.5 Max Value -1.5

3.2.3 Interactivity: saving conclusions as you browse

The picture is interactive and you clicking on different items will offer you different options.

If you click on a gene, you'll be offered (see picture below) to either consult the gene summary page or define this gene as a target gene of a regulatory region.



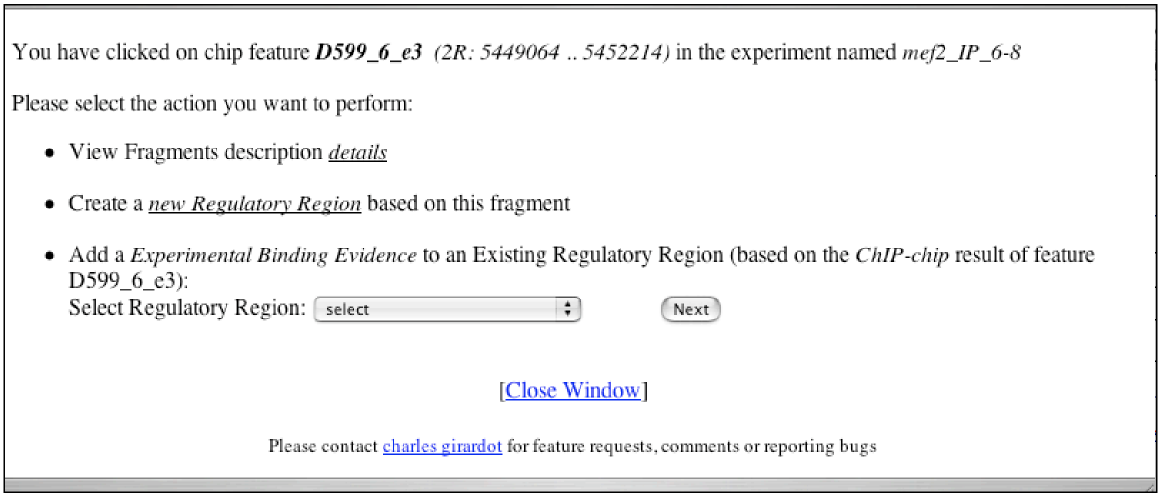
You have clicked on Gene ***CG10379 (3R: 19607508 .. 19627396)***
Please select the action you want to perform:

- View Gene description details *choose*
- Define *CG10379* as a *target gene* of an existing Regulatory Region
  Select Regulatory Region: none Next

[Close Window]

Please contact charles girardot for feature requests, comments or reporting bugs

If you click on a tiling array feature, you'll be offered (see picture below) to consult the feature summary page, define a new regulatory region based on this fragment or add this experimental results as a supporting evidence of an existing regulatory region (this option is offered only if the feature you selected overlaps with exiting regions).



You have clicked on chip feature ***D599_6_e3*** *(2R: 5449064 .. 5452214)* in the experiment named *mef2_IP_6-8*

Please select the action you want to perform:

- View Fragments description *details*
- Create a *new Regulatory Region* based on this fragment
- Add a *Experimental Binding Evidence* to an Existing Regulatory Region (based on the *ChIP-chip* result of feature D599_6_e3):
  Select Regulatory Region: select Next

[Close Window]

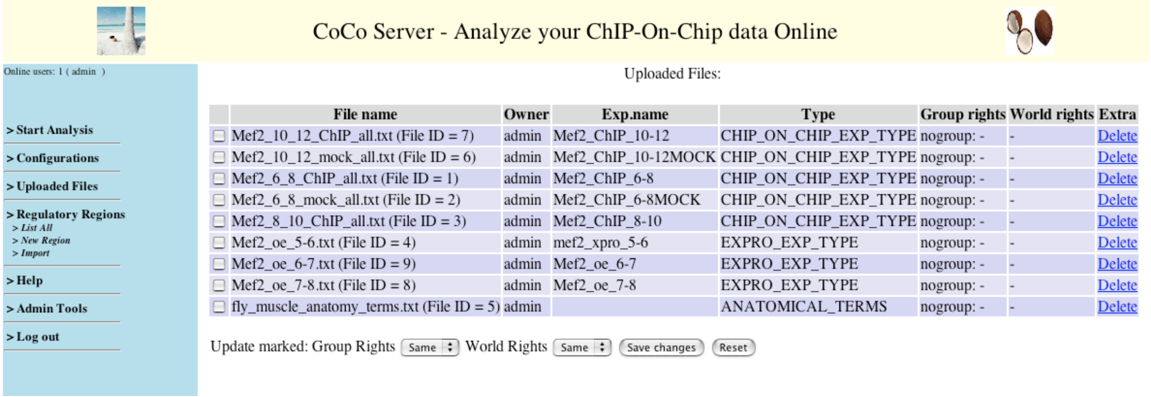
Please contact charles girardot for feature requests, comments or reporting bugs

4. Managing your files

Once files have been uploaded to the server (as you create configurations), they are stored and made available in drop down selection boxes. You can see your files using the "Uploaded Files" menu. There you can delete or share your files with colleagues.

Three sharing levels are available: no sharing, group sharing and world sharing (world meaning here people that have login).

In the list of files, you see both yours and the one you have access to. Deleting a file can be done only if this file is not used by existing configurations. This limitation is made because it is sometimes (e.g. at software upgrade time) necessary to recomputed configurations.



CoCo Server - Analyze your ChIP-On-Chip data Online

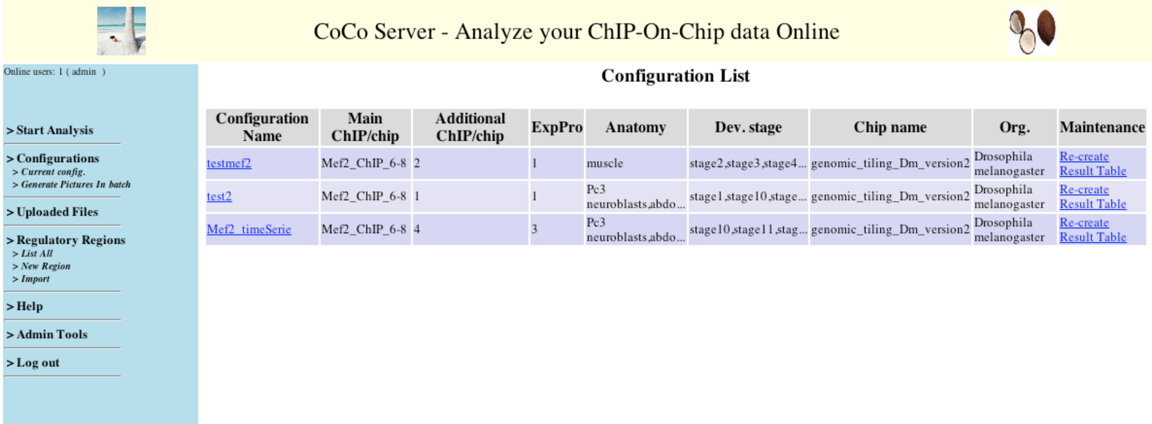
Uploaded Files:

	File name	Owner	Exp.name	Type	Group rights	World rights	Extra
□	Mef2_10_12_ChIP_all.txt (File ID = 7)	admin	Mef2_ChIP_10-12	CHIP_ON_CHIP_EXP_TYPE	nogroup: -	-	Delete
□	Mef2_10_12_mock_all.txt (File ID = 6)	admin	Mef2_ChIP_10-12MOCK	CHIP_ON_CHIP_EXP_TYPE	nogroup: -	-	Delete
□	Mef2_6_8_ChIP_all.txt (File ID = 1)	admin	Mef2_ChIP_6-8	CHIP_ON_CHIP_EXP_TYPE	nogroup: -	-	Delete
□	Mef2_6_8_mock_all.txt (File ID = 2)	admin	Mef2_ChIP_6-8MOCK	CHIP_ON_CHIP_EXP_TYPE	nogroup: -	-	Delete
□	Mef2_8_10_ChIP_all.txt (File ID = 3)	admin	Mef2_ChIP_8-10	CHIP_ON_CHIP_EXP_TYPE	nogroup: -	-	Delete
□	Mef2_oe_5-6.txt (File ID = 4)	admin	mef2_xpro_5-6	EXPRO_EXP_TYPE	nogroup: -	-	Delete
□	Mef2_oe_6-7.txt (File ID = 9)	admin	Mef2_oe_6-7	EXPRO_EXP_TYPE	nogroup: -	-	Delete
□	Mef2_oe_7-8.txt (File ID = 8)	admin	Mef2_oe_7-8	EXPRO_EXP_TYPE	nogroup: -	-	Delete
□	fly_muscle_anatomy_terms.txt (File ID = 5)	admin		ANATOMICAL_TERMS	nogroup: -	-	Delete

Update marked: Group Rights Same World Rights Same

5. Managing your configurations

You can see your configurations using the "Configurations" menu. There you can view details or delete your configurations. Note that sharing configurations is not yet possible. We encourage users to delete old configurations as configurations occupy quite some space on the server.

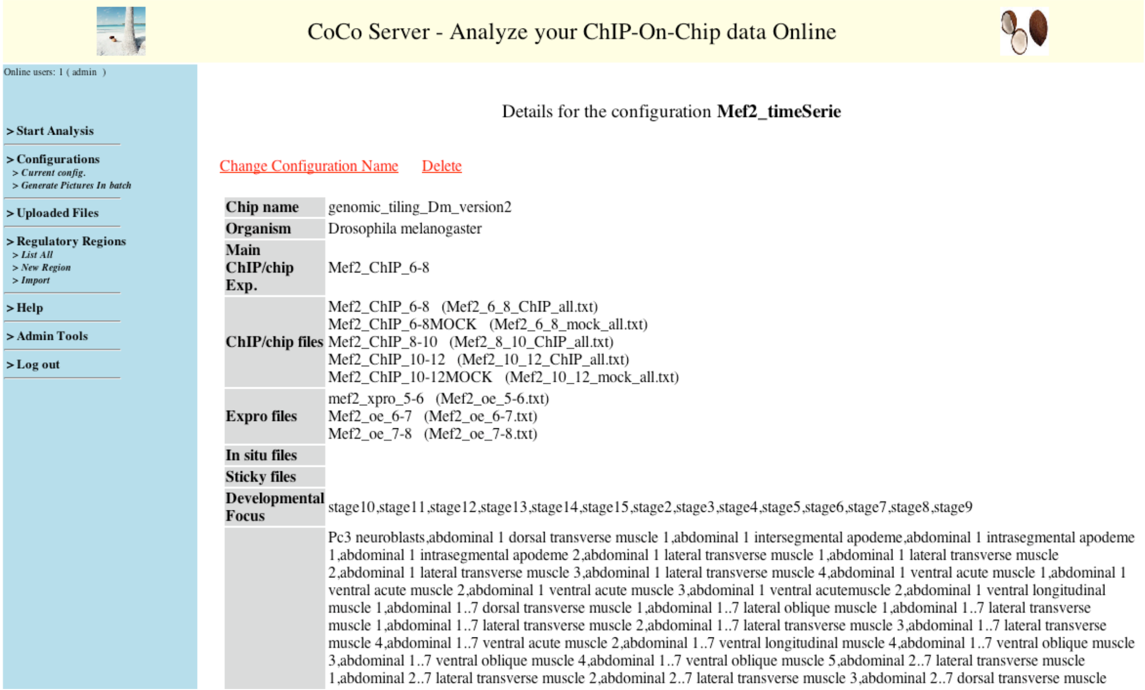


CoCo Server - Analyze your ChIP-On-Chip data Online

Configuration List

Configuration Name	Main ChIP/chip	Additional ChIP/chip	ExpPro	Anatomy	Dev. stage	Chip name	Org.	Maintenance
testmef2	Mef2_ChIP_6-8	2	1	muscle	stage2,stage3,stage4...	genomic_tiling_Dm_version2	Drosophila melanogaster	Re-create Result Table
test2	Mef2_ChIP_6-8	1	1	Pc3 neuroblasts,abdo...	stage1,stage10,stage...	genomic_tiling_Dm_version2	Drosophila melanogaster	Re-create Result Table
Mef2_timeSerie	Mef2_ChIP_6-8	4	3	Pc3 neuroblasts,abdo...	stage10,stage11,stag...	genomic_tiling_Dm_version2	Drosophila melanogaster	Re-create Result Table

Clicking on a configuration name brings you to the detailed Configuration view shown on the picture below.



Details for the configuration **Mef2_timeSerie**

Chip name	genomic_tiling_Dm_version2
Organism	Drosophila melanogaster
Main ChIP/chip Exp.	Mef2_ChIP_6-8
ChIP/chip files	Mef2_ChIP_6-8 (Mef2_6_8_ChIP_all.txt) Mef2_ChIP_6-8MOCK (Mef2_6_8_mock_all.txt) Mef2_ChIP_8-10 (Mef2_8_10_ChIP_all.txt) Mef2_ChIP_10-12 (Mef2_10_12_ChIP_all.txt) Mef2_ChIP_10-12MOCK (Mef2_10_12_mock_all.txt)
Expro files	mef2_xpro_5-6 (Mef2_oe_5-6.txt) Mef2_oe_6-7 (Mef2_oe_6-7.txt) Mef2_oe_7-8 (Mef2_oe_7-8.txt)
In situ files	
Sticky files	
Developmental Focus	stage10,stage11,stage12,stage13,stage14,stage15,stage2,stage3,stage4,stage5,stage6,stage7,stage8,stage9
	Pc3 neuroblasts,abdominal 1 dorsal transverse muscle 1,abdominal 1 intersegmental apodeme,abdominal 1 intrasegmental apodeme 1,abdominal 1 intrasegmental apodeme 2,abdominal 1 lateral transverse muscle 1,abdominal 1 lateral transverse muscle 2,abdominal 1 lateral transverse muscle 3,abdominal 1 lateral transverse muscle 4,abdominal 1 ventral acute muscle 1,abdominal 1 ventral acute muscle 2,abdominal 1 ventral acute muscle 3,abdominal 1 ventral acutemuscle 2,abdominal 1 ventral longitudinal muscle 1,abdominal 1..7 dorsal transverse muscle 1,abdominal 1..7 lateral oblique muscle 1,abdominal 1..7 lateral transverse muscle 1,abdominal 1..7 lateral transverse muscle 2,abdominal 1..7 lateral transverse muscle 3,abdominal 1..7 lateral transverse muscle 4,abdominal 1..7 ventral acute muscle 2,abdominal 1..7 ventral longitudinal muscle 4,abdominal 1..7 ventral oblique muscle 3,abdominal 1..7 ventral oblique muscle 4,abdominal 1..7 ventral oblique muscle 5,abdominal 2..7 lateral transverse muscle 1,abdominal 2..7 lateral transverse muscle 2,abdominal 2..7 lateral transverse muscle 3,abdominal 2..7 dorsal transverse muscle

6. Defining Regulatory Regions And Target Genes

The primary goal of CoCo is to help users in finding regulatory regions and defining their target genes. Regulatory region boundaries definition and target gene assignment represent very valuable knowledge. As we'll explain, CoCo provides different means to realize these tasks and provides a detailed model to store them. To fully benefit from these data, even years after decision has been made, it is important to know how scientists have come to their conclusions. CoCo addresses this issue by letting users give confidence to their conclusions and attaching experimental evidences. For example, CoCo automatically records evidences about the ChIP-on-chip results used to initiate a regulatory region definition. These features allow users to accumulate conclusions about regulatory regions over time and should ensure reusability.

CoCo offers different ways to create regulatory regions:

1. Import from file
2. Create a region from scratch (e.g. that you found in literature)
3. While browsing your results as explained before

6.1 Regulatory region model in CoCo

In CoCo, a regulatory region is not only defined as a genomic location. Here is the model used in CoCo.

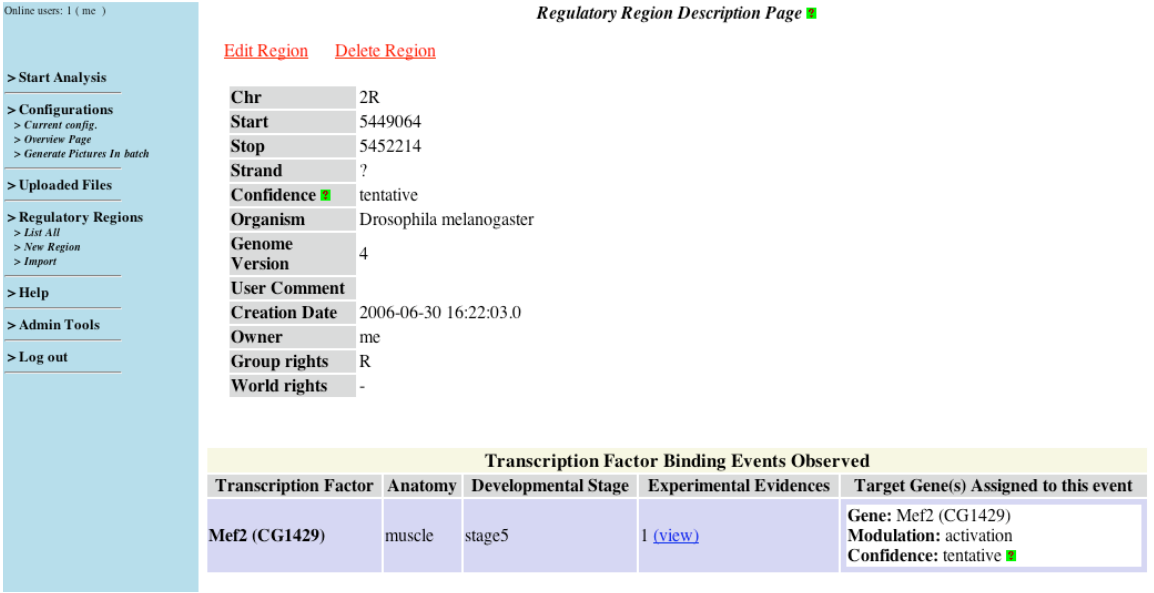
A ***regulatory region*** (RR) is a genomic region where ***transcription factor(s)*** (TF) bind to the genome. Thus a region doesn't only describe a single binding site but a regulatory module. The fact that a TF binds to a ***regulatory region*** is referred to as a ***binding event*** that occurs in specific ***spatio-temporal*** conditions. A ***regulatory region*** can have many ***binding events*** described for multiple TFs. When describing a ***binding event*** for a given TF, you can optionally specify ***spatio-temporal*** conditions, the exact binding site boundaries and a ***target gene***. In addition, binding events can be supported by experimental ***evidence(s).***

A target gene is a gene which expression is affected by a ***binding event.*** Because a regulatory region may be associated with many binding events, it may have many target genes. Actually, CoCo allows you to associate or ***assign*** more than one target gene to a binding event (and by extension to a region). This is especially useful in situations where it is unclear what gene(s) are affected by the binding event.

Confidence values are attached to both the regulatory region and target gene assignment. Different values are available reflecting different level of confidence:

- *Tentative* is the lowest confidence level and indicates ...well a possibility
- *Predictive* comes after tentative and indicates that the conclusion comes from a prediction tool (that's the real difference between tentative and predictive)
- *Confirmed* indicates that the region or the target gene assignment has been experimentally confirmed (experimental evidence should be available)
- *Reviewed* indicates that the region or the target gene assignment has been published in the literature

The picture below shows an example of a (unreal) regulatory region created while browsing experimental data.



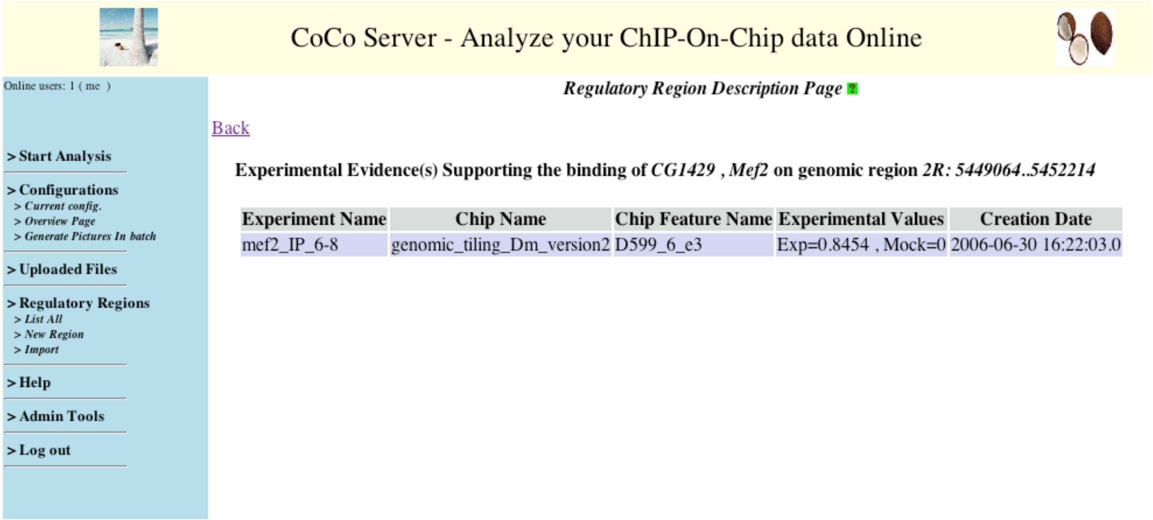
Regulatory Region Description Page ?

Chr	2R
Start	5449064
Stop	5452214
Strand	?
Confidence ?	tentative
Organism	Drosophila melanogaster
Genome Version	4
User Comment	
Creation Date	2006-06-30 16:22:03.0
Owner	me
Group rights	R
World rights	-

Transcription Factor Binding Events Observed

Transcription Factor	Anatomy	Developmental Stage	Experimental Evidences	Target Gene(s) Assigned to this event
Mef2 (CG1429)	muscle	stage5	1 (view)	**Gene:** Mef2 (CG1429) **Modulation:** activation **Confidence:** tentative ?

From the above picture, you can see that the region has one binding event defined for “mef2”. The column “Experimental Evidences” indicates that the binding event is supported by one experimental evidence. If you click the link, a page displaying evidence details supporting this binding event (and by extension the regulatory region) is shown. In this example, the evidence recorded the experiment, chip feature ID and feature enrichment values.



Experimental Evidence(s) Supporting the binding of *CG1429 , Mef2* on genomic region *2R: 5449064..5452214*

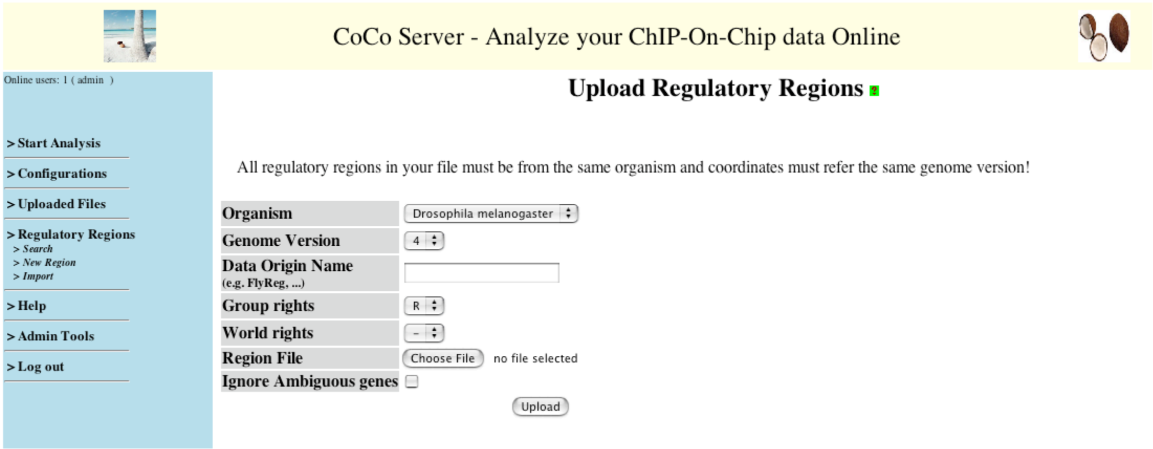
Experiment Name	Chip Name	Chip Feature Name	Experimental Values	Creation Date
mef2_IP_6-8	genomic_tiling_Dm_version2	D599_6_e3	Exp=0.8454 , Mock=0	2006-06-30 16:22:03.0

6.2 Importing regions into CoCo

You can import regulatory regions in bunch. To do this you must first assemble a tab-delimited file (see format below) and then choose "*Import*" in the "*Regulatory Region*" menu. The picture below depicts the import page.

In addition to the file containing region definitions, you have to provide:

1. The **organism**
2. The **genome version**: the genome version the region coordinates found in the file refer to
3. A **Data Origin Name**: all regions will be attached to this origin. This is quite important as origins can be used to filter regions later on. Here you should indicate where you got these regions from e.g. Flyreg…
4. The **group/world rights**: if you don't share these regions, other people won't see them. If you come with predictions, you certainly don't want to share them. Alternatively, if regions origin is literature, we encourage you to share them.
5. **Ignore Ambiguous Gene** option can be use to ignore lines for which gene symbol (relative to either transcription factor or target genes) can't be uniquely resolved, i.e. the symbol either has no match in CoCo db or maps to multiple genes. We advise to first NOT use this option. This will cause CoCo to either successfully upload all regions or print a report about ambiguous genes. In this latter case, no regions will be uploaded and you can either decide to correct your file or re-run using the **Ignore Ambiguous Gene** option on (regions with ambiguous genes will be ignored).



Upload Regulatory Regions

All regulatory regions in your file must be from the same organism and coordinates must refer the same genome version!

Organism	Drosophila melanogaster
Genome Version	4
Data Origin Name (e.g. FlyReg, ...)	
Group rights	R
World rights	-
Region File	Choose File no file selected
Ignore Ambiguous genes	□

Upload

File Format

The file format is quite extensible and allows you to give extensive details or not. Here is the complete list of columns that can be found in the file (column order doesn't matter). Note that there are both mandatory and optional columns and that providing some columns imply that you provide others (i.e. optional columns might become mandatory)

Important: headers must appear as the first valid line in the file. Comment lines, i.e. line starting with "#" can be found prior to the header line. Headers must be written as described and must respect the case.

```
# comments
# more comments
region_chr	region_start	region_stop	[more tab-delimited columns with headers…]
```

Mandatory headers:

- ***region_chr*** : the regulatory region chromosome
- ***region_start*** : the regulatory region start
- ***region_stop*** : the regulatory region stop

As a minimum file, CoCo will accept a file containing these 3 columns only though it is really not informative to load such regions...

Optional headers:

- ***region_strand*** : if applicable, the region strand.
- ***region_confidence*** : the confidence you have about this region to be real. One of "tentative", "predictive", "confirmed", "reviewed". If not provided, it is defaulted to "tentative"

- ***region_comment*** : a free description about the region, use this field to store e.g literature information
- ***tf*** : a transcription factor binding to the region
- ***tf_binding_anatomy*** : the anatomical part(s) where the binding occurs ; comma-separated values accepted to specify multiple anatomical parts
- ***tf_binding_stage*** : the stage(s) at which the binding occurs ; comma-separated values accepted to specify multiple stages
- ***tf_binding_site_start*** : the transcription factor binding site start ; in case you know where exactly the transcription factor binds within the region (i.e. its binding site), you can precise it
- ***tf_binding_site_stop*** : the transcription factor binding site stop (see above)
- ***tf_binding_site_strand*** : the transcription factor binding site strand (see above)
- ***target_gene*** : the gene which expression is modulated by the transcription factor in the given spatio-temporal conditions
- ***target_gene_modulation*** : the type of expression modulation , one of "activation", "repression", "unknown"
- ***target_gene_confidence*** : the confidence to give to the gene assignment. One of "tentative", "predictive", "confirmed", "reviewed"
- ***target_gene_comment*** : a free description about the gene assignment
- ***target_gene_evidence*** : a free comment about evidences supporting this assignment

If the assignment comes from a prediction (i.e. bioinformatics) tool, the following fields can be provided:

- ***target_gene_prediction_origin*** : the name of the prediction tool, together with parameters used
- ***target_gene_prediction_score*** : a score for the gene assignment, if available
- ***target_gene_prediction_score_type*** : if a score is provided , its type i.e. a small (255 char) description of the score meaning e.g. "p_value", ...

As explained, dependencies between fields occur:

- ***tf_**** fields should be found ONLY if the "***tf***" field is described.
- ***target_gene_**** fields should be found ONLY if the "***target_gene***" field is described AND if "tf" is described.
- ***tf_binding_site_start*** and ***tf_binding_site_stop*** should be both provided or both empty. If ***tf_binding_site_strand*** is provided, both ***tf_binding_site_start*** and ***tf_binding_site_stop*** should be provided
- If ***target_gene_prediction_**** field(s) are provided, ***target_gene_prediction_origin*** is mandatory

Regulatory region creation rules when importing regions:

1. If multiple lines hold the same region_chr/region_start/region_stop, CoCo creates a unique regulatory region but will use all lines to create binding events and target genes

2. tf_binding_stage and tf_binding_anatomy accept comma separated values. If you chose to use this, a binding event will be create for each possible combination. If this is not reflecting reality, you must duplicate lines as needed. For example, if the TF mef2 binds a region 2R:100-200 at stage1 in the heart, stage2 in the heart and stage3 in both the visceral muscle and the heart you must define 2 lines, e.g. (only relevant headers are shown):

region_chr	region_start	region_stop	tf	tf_binding_anatomy	tf_binding_stage
2R	100	200	Mef2	heart	stage1,stage2
2R	100	200	Mef2	visceral muscle,heart	stage3

The region defined in this example will end up with 4 binding events.

3. If you describe a target gene in the line, it will be added as a target gene to all binding events described in the line. From the previous example, let's assume that the two lines have the value "twist" in the column "target_gene" => the four binding events will have "twist" as a target gene but as 2 distinct relationships. Indeed, the first line could describe an *activation* type "target_gene_modulation" while the second a *repression* gene modulation type.
4. To associate multiple target genes to the same binding event. You should follow the same strategy as described in 2)

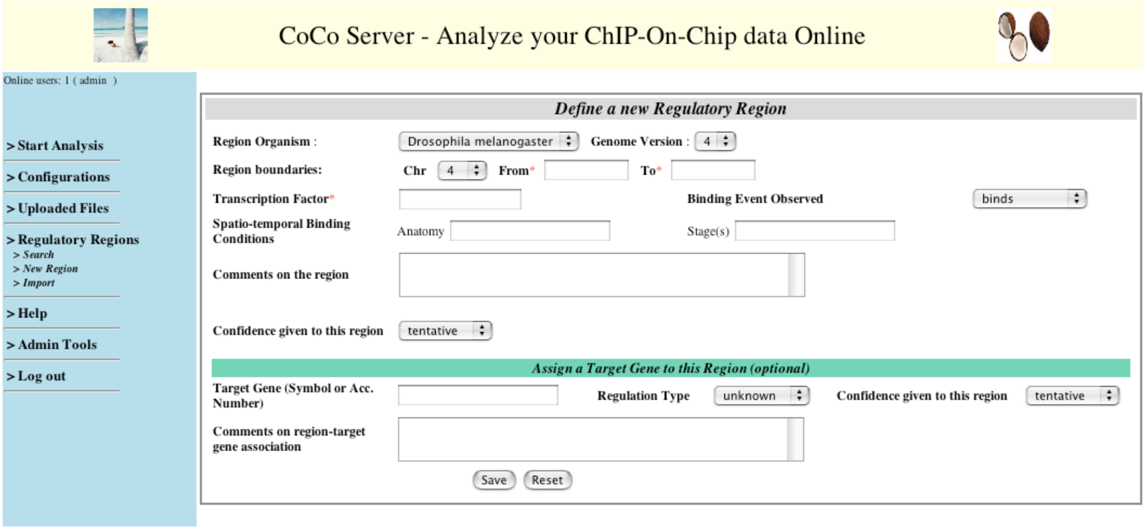
6.3 Creating a region from scratch

You can define a new regulatory region using the "New Region" sub-menu of "Regulatory Regions" menu. Simply fill in the form (see example below). If your CoCo installation supports multiple organisms, changing the *organism* value will update both the *genome version* and *chromosome* lists.
The form is split in two section:

1. Regulatory Region Boundaries and Binding Condition: this section holds values to define both the regulatory region and the Transcription Factor binding event. This section must be filled
2. Target Gene Assignment: here you can define a target gene of the region.

Mandatory fields are marked with a red star. Note that the spatio-temporal conditions are not mandatory as it is sometimes hard to find this information (published regions) but we encourage you to fill these fields whenever you can.

As usual, in the *Transcription Factor* (and *Target Gene*) fields, simply specify the official symbol or gene accession (ask your administrator about which database accession you can use). CoCo will anyway complain if it can't map uniquely your input to the database.



Define a new Regulatory Region

Region Organism : Drosophila melanogaster Genome Version : 4

Region boundaries: Chr 4 From* To*

Transcription Factor* Binding Event Observed binds

Spatio-temporal Binding Conditions Anatomy Stage(s)

Comments on the region

Confidence given to this region tentative

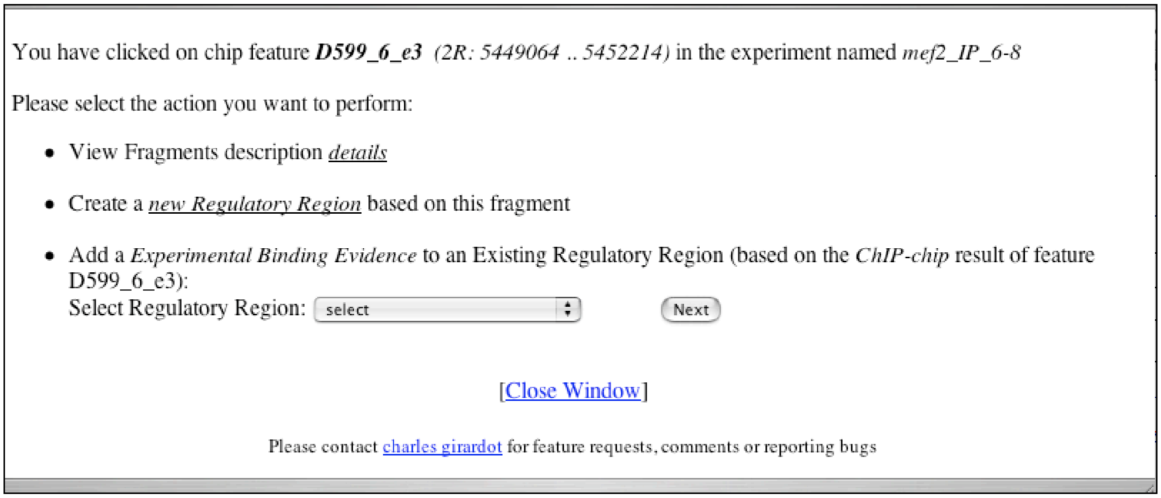
Assign a Target Gene to this Region (optional)

Target Gene (Symbol or Acc. Number) Regulation Type unknown Confidence given to this region tentative

Comments on region-target gene association

6.4 Creating a region and adding experimental evidences while browsing experimental data

While browsing your experiments, you can click on chip feature. This will open up an option window that allows you to perform…well different actions. One of them is named “Create a new regulatory region based on this fragment” as shown on the picture below.



You have clicked on chip feature ***D599_6_e3*** *(2R: 5449064 .. 5452214)* in the experiment named *mef2_IP_6-8*

Please select the action you want to perform:

- View Fragments description *details*
- Create a *new Regulatory Region* based on this fragment
- Add a *Experimental Binding Evidence* to an Existing Regulatory Region (based on the *ChIP-chip* result of feature D599_6_e3):
  Select Regulatory Region: select Next

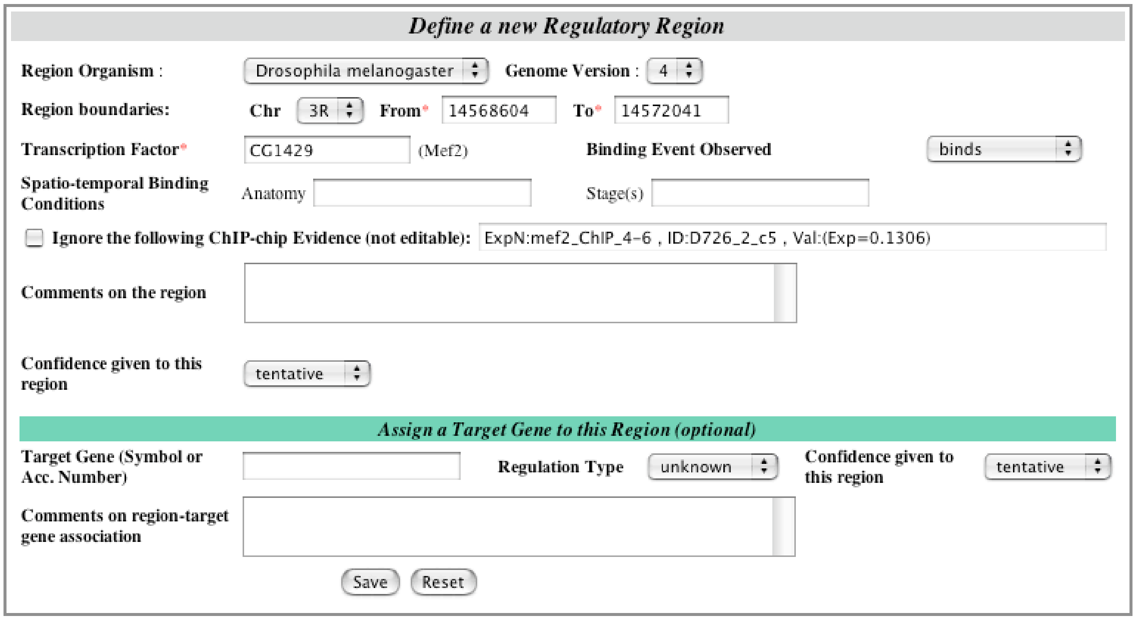
[Close Window]

Please contact charles girardot for feature requests, comments or reporting bugs

If you follow this option, the regulatory region definition page will show up. This page (see picture below) is similar to the regulatory region creation accept that:

1. the form is pre-filled with region boundaries corresponding to the chip feature boundaries

2. the form is pre-filled with the transcription factor accession concerned by the current configuration
3. the form exhibits an experimental evidence. The cryptic text can’t be modified. By default the evidence will be added to the binding event (that is created at the same time the region is created) unless you select the checkbox in front.
4. The region origin can’t be modified and is set to “Experimental”



Define a new Regulatory Region

Region Organism : Drosophila melanogaster   Genome Version : 4

Region boundaries: Chr 3R   From* 14568604   To* 14572041

Transcription Factor* CG1429 (Mef2)   Binding Event Observed binds

Spatio-temporal Binding Conditions   Anatomy   Stage(s)

□ Ignore the following ChIP-chip Evidence (not editable): ExpN:mef2_ChIP_4-6 , ID:D726_2_c5 , Val:(Exp=0.1306)

Comments on the region

Confidence given to this region tentative

Assign a Target Gene to this Region (optional)

Target Gene (Symbol or Acc. Number)   Regulation Type unknown   Confidence given to this region tentative

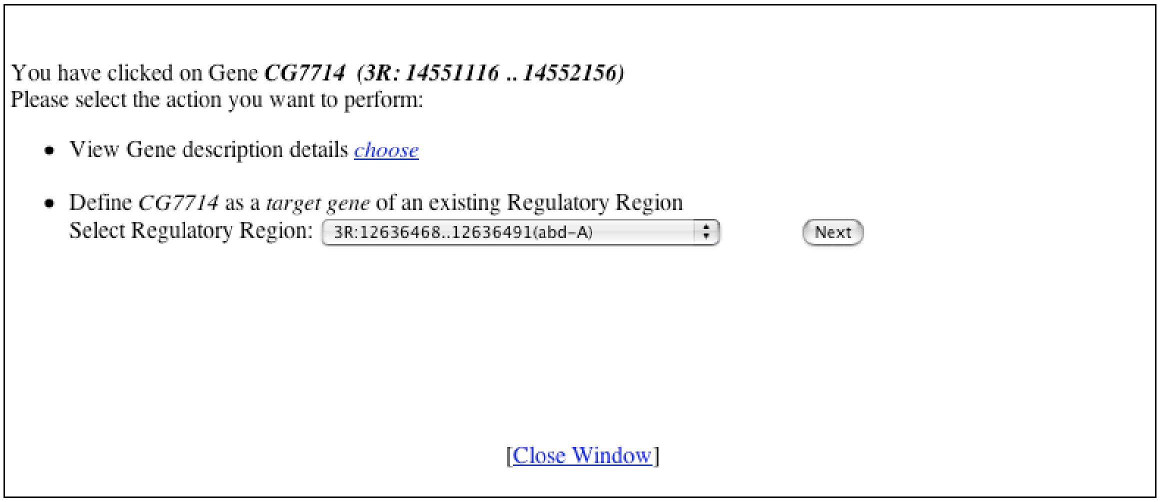
Comments on region-target gene association

[Close Window]

Another option offered in the option window is “*Add an experimental evidence…*” and let you attach the selected feature’s experimental results as an evidence supporting the binding of a TF onto an existing region. Indeed, this option will be offered only if the selected feature overlaps with existing regions. Simply select the right region from the drop down menu and complete the wizard.

6.5 Assigning target genes to regions while browsing experimental data

As you browse your results, clicking on a gene will offer you the possibility to assign this selected gene to existing regions as shown on the picture below.
Note that the regions proposed in the drop down menu are those found in the current genomic region i.e. in the picture you clicked on.



You have clicked on Gene ***CG7714 (3R: 14551116 .. 14552156)***
Please select the action you want to perform:

- View Gene description details *choose*
- Define *CG7714* as a *target gene* of an existing Regulatory Region
  Select Regulatory Region: 3R:12636468..12636491(abd-A) Next

[Close Window]

6.6 Searching for regulatory regions

The “Search” sub-menu of the “Regulatory Regions” menu lets you list regulatory regions stored in CoCo. As you click on the link, no regions will be displayed and a message will invite you to position filtering criteria as shown on the picture below.

Note: regions are displayed for a unique organism.
Note 2: only regions on which you have read rights will be displayed.

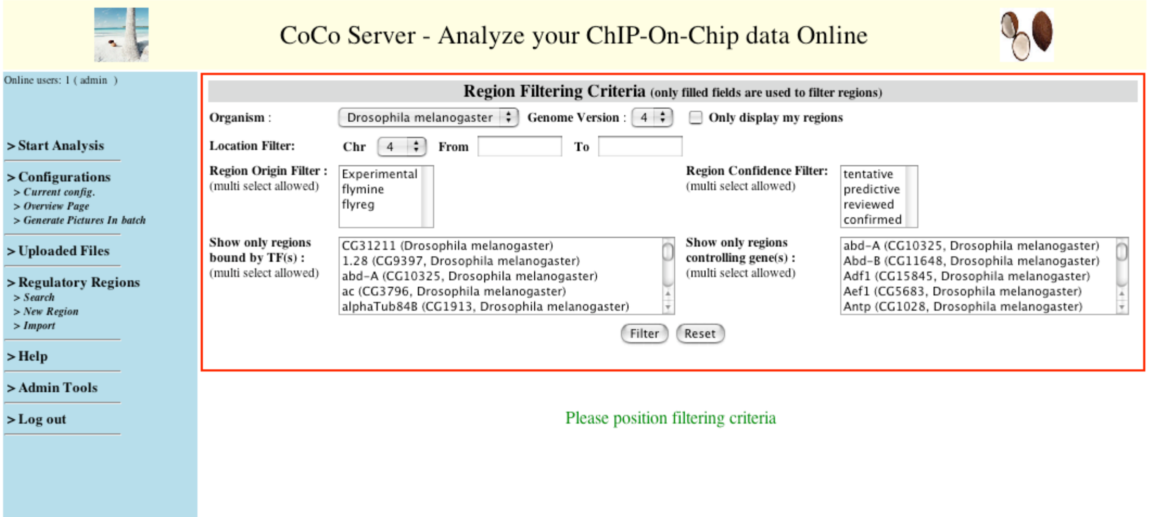
CoCo offers an extensive filtering interface. It works the following way:

- To view all regions, don’t fill any filtering fields and simply click on “Filter”
- To filter on some criteria, fill the appropriate field. Don’t position other fields and they won’t be used to filter

Filtering options:

- **Genomic location**: only regions that are fully included in the specified boundaries will be shown
- **Region Origin**: when used, only regions from selected origins are displayed. Proposed origins are those for which you have read rights on at least one region. Note that the “Experimental” origin is always proposed.
- **Region Confidence**: when used, only regions with selected confidence are displayed
- **Only display my regions**: when checked, only regions that you created (owned by you) are displayed
- **Transcription Factor Filter**: here you can select transcription factor and only display regions bound by these. Note that all TFs proposed in the list are TFs that bind at least one region defined in CoCo. Unfortunately, you might not have the read right on these regions.

- **Target Gene Filter**: here you can define a gene list and display only regions that have one of these genes as target gene. Note that all proposed genes in the list are target genes defined in CoCo. Unfortunately, you might not have the read right on these regions.



Region Filtering Criteria (only filled fields are used to filter regions)

Organism : Drosophila melanogaster Genome Version : 4 □ Only display my regions

Location Filter: Chr 4 From To

Region Origin Filter : (multi select allowed)
Experimental
flymine
flyreg

Region Confidence Filter: (multi select allowed)
tentative
predictive
reviewed
confirmed

Show only regions bound by TF(s) : (multi select allowed)
CG31211 (Drosophila melanogaster)
1.28 (CG9397, Drosophila melanogaster)
abd-A (CG10325, Drosophila melanogaster)
ac (CG3796, Drosophila melanogaster)
alphaTub84B (CG1913, Drosophila melanogaster)

Show only regions controlling gene(s) : (multi select allowed)
abd-A (CG10325, Drosophila melanogaster)
Abd-B (CG11648, Drosophila melanogaster)
Adf1 (CG15845, Drosophila melanogaster)
Aef1 (CG5683, Drosophila melanogaster)
Antp (CG1028, Drosophila melanogaster)

Please position filtering criteria

7. Help in CoCo

CoCo has three help resources:

1. this document
2. help displayed on you move your mouse over e.g. form fields
3. little help icons are usually present at every page. Clicking them will open up a context sensitive help window

8. File Formats

8.1 ChIP-chip Data File

CoCo accepts ChIP-chip results as a tab-delimited file holding 2 columns:

- the first must hold a feature ID
- the second a experimental value. What you provide here is up to you but the value should certainly be some kind of enrichment value or statistical score reflecting the likelihood that this feature is enriched.

8.2 In Situ Data File

CoCo accepts In Situ results as a tab-delimited file holding 3 columns:

- Column 1: GeneSymbol
- Column 2: dev_stage
- Column 3: anatomy

8.3 Expression Profiling Data File

CoCo accepts Expro results as a tab-delimited file holding 2 columns:

- the first must hold a feature Id or gene symbol/accession
- the second a experimental value. What you provide here is up to you but the value should certainly be some kind of expression value (e.g. log-ratio) or statistical score reflecting the likelihood that this feature/gene is differentially expressed.

8.4 Term List File

Term lists are provided as simple file with a unique term per line, only one column.

8.5 Sticky File

Sticky feature lists are provided as simple file with a unique feature ID per line, only one column.

8.6 Regulatory Region File

Please see chapter 6.

8.7 Genome Annotations

CoCo uses GFFv3 file format (http://flybase.bio.indiana.edu/annot/gff3.html) to define genome annotations. Particularly important aspect is what you put in the last column:

- CoCo uses the "ID" attribute of gene annotations to create gene symbol. In CoCo, gene symbol must be unique. Every gene annotation must have an ID attribute.
- If present, CoCo uses the "Name" attribute of gene annotations to create gene names
- If present, CoCo uses the "synonym" attribute to create synonyms. Note that the value of the "synonym" attribute can specify a comma-separated list of synonyms. CoCo will register each synonym in the gene synonym table.

9. Supported Arrays – note about high density arrays

CoCo virtually supports all arrays as long as the server running CoCo has enough memory and users are patient enough! In practice, we have observed acceptable compute times with arrays up to 100K. This means that the ~6M Affymetrix tiling arrays will certainly not work well with the current release of CoCo on small servers (e.g. ~1Go memory).

As we are starting to use Affymetrix arrays, we are willing to address this issue soon so you should keep checking the coco download page for new release in the next months.

9.1 Technical considerations

In the current version of CoCo, each tiling array must have a clone map file (a file containing the genomic coordinates of each feature found on the tiling array) that is used to find feature positions at configuration creation. This mapping is performed by loading the whole map in server memory. This results in high memory requirement for high-density arrays and pretty long processing time to create a configuration. That's the first technical limitation. The second limitation would then be the time required to assemble each picture on user request. Indeed, CoCo starts a new R process for each picture generation. Thus the time to build a picture is the sum of the time for R to start + the time for R to load all data integrated in the configuration + the time for R to generate the picture + the time for R to shutdown. We are thinking about using a solution like Rserve to cut down the picture generation time. This should be a first step to proper Affymetrix dataset handling. But still, loading datasets of 6M point each will require a pretty big server for picture generation (several Go of RAM).

9.2 Other considerations

Besides technical aspects, we are not sure that displaying all features of an e.g. an Affymetrix chip is a very likely use case. CoCo has been initially designed to cope with small/medium size tiling arrays. These arrays are usually made with clones ranging from 500b to several Kb. The pictures generated by CoCo accommodate perfectly with these sizes of feature: you can zoom out and display genomic regions of several Mb and still see tiling array features. With oligo-based tiling arrays i.e. from 25 (Affymetrix) to 60 (NimbleGen) bases, zooming out becomes quickly useless. Indeed, we observe that users like to display from 30 to 100 Kb around enriched features to have a good overview of the surrounding genomic environment. Unfortunately, oligo features of 60 bases become invisible when displaying more than 30 Kb. The examples below show views obtained with a NimbleGen chip (~380K features). In addition, the common approach when analyzing results from high density arrays is to run a region discovery algorithm like

MAT (http://chip.dfci.harvard.edu/~wli/MAT/) or TileMap (http://biogibbs.stanford.edu/~jihk/TileMap/index.htm) after normalization and look at enriched regions directly. We think that the best approach would then be to allow users to upload regions directly. This latest approach is certainly quite easy to implement and we'll implement this soon in CoCo.

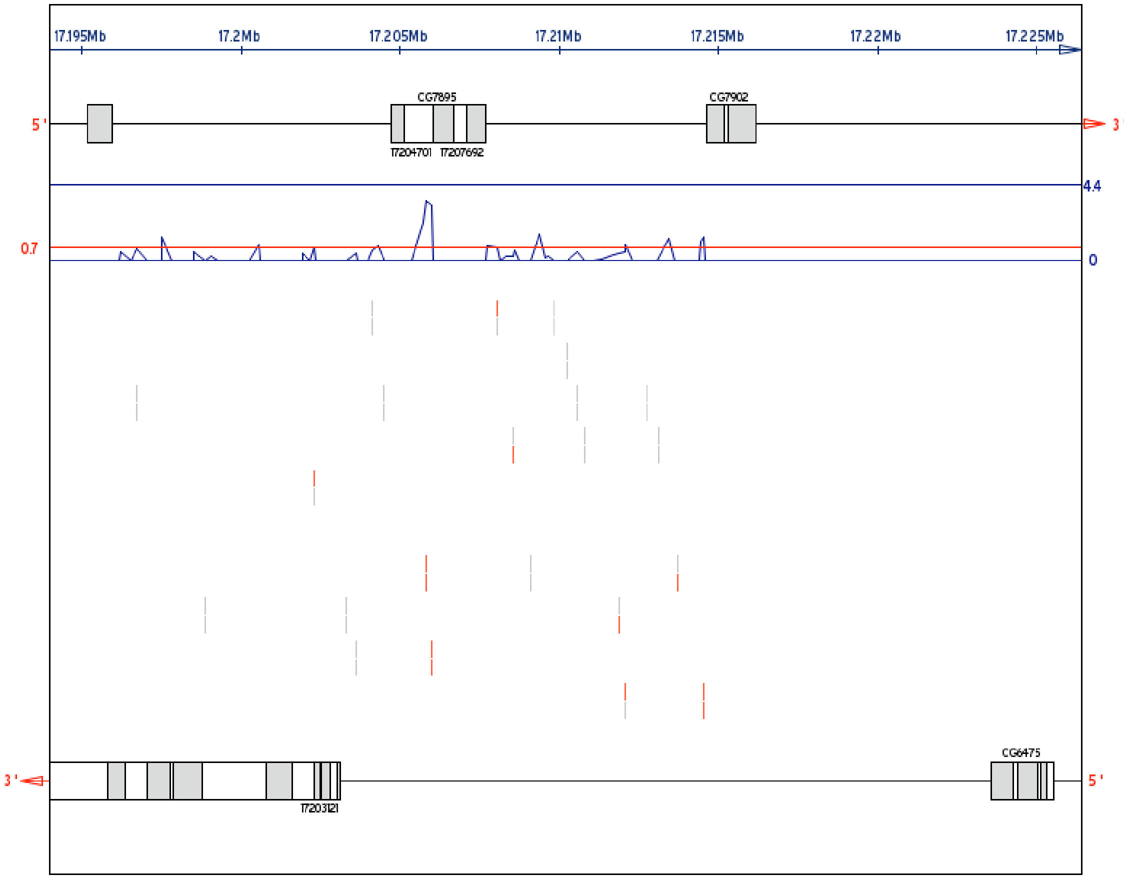


Figure 1. Display of a 30Kb Region Using a NimbleGen Tiling Array

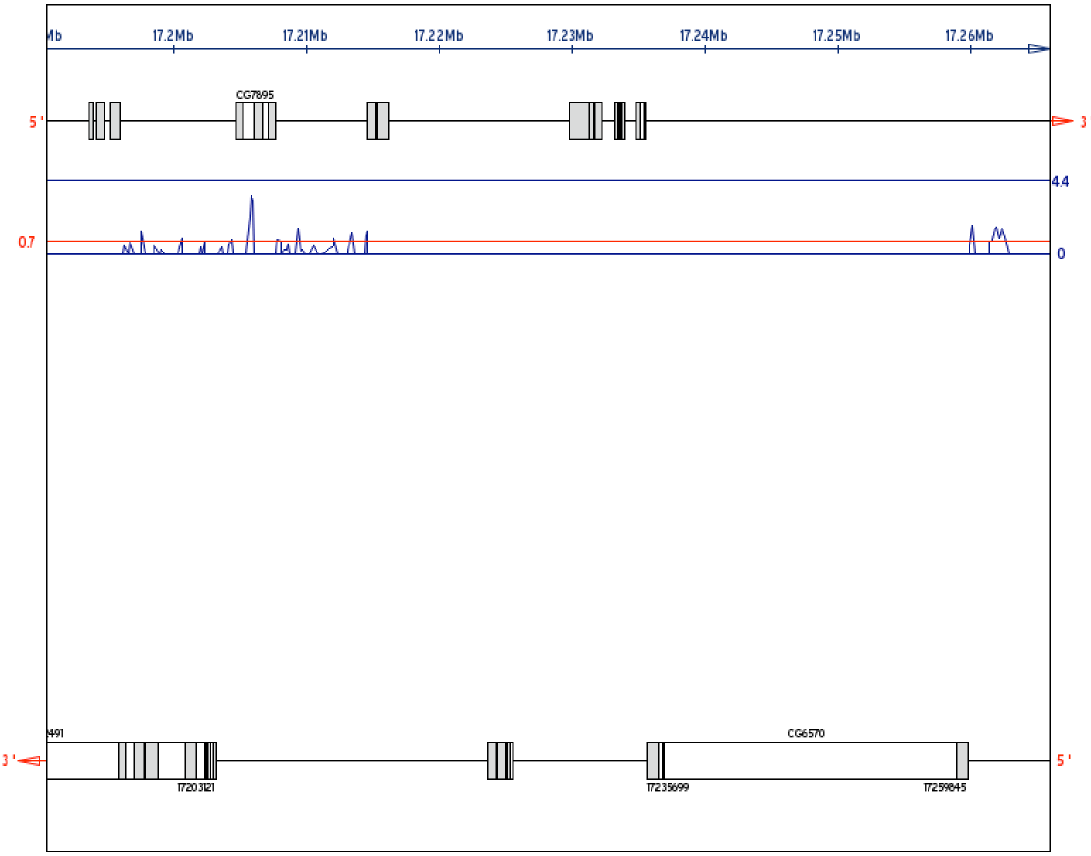


Figure 2. Display of a 70 Kb Region using a NimbleGen Tiling Array

9.3 What you can do with the current CoCo version

If you want to upload results from high density arrays in CoCo, here is what you can do (and what we did for NimbleGen array):

1. Define the chip as usual. In case you don't use the MM probes (Affymetrix chips), don't put them in the clone map. This will save 3M lines and lots of compute time.
2. Pre-process your chip-chip files: as you build them for CoCo, remove all those probes that are clearly in the background by applying some filtering of your choice. Indeed, these feature results are useless and they will only increase compute time. Filtering could be: (1) keep only features that belong to enriched regions i.e. as defined by TileMap or MAT , (2) remove all features having a enrichment ratio less than e.g. 0.3.

3. You can rescan the clone map file and keep only those features that appear in any of your result files.
4. If you want to have a nice display, make sure that all your chip-chip datasets (after filtering) contain the same set of features by adding back features found in one dataset but not in others.
5. Contact Girardot@embl.de, I should have perl scripts you can adapt that do steps 2 and 4!

CoCo installation and maintenance document

CoCo is a JAVA web application using R (http://lib.stat.cmu.edu/R/CRAN/) for statistics and image generation. Data are stored in a MySQL (www.mysql.com) InnoDb database.

1. Pre-requisites

CoCo can be downloaded from http://furlonglab.embl.de/methods/tools/coco

CoCo needs the following third party products to be installed **prior** to CoCo installation. All these third-party products are well established

- JAVA >= 1.4.2 available
- Ant available (>= 1.5)
- Tomcat installed (tested with 5.0.* version)
- R version 2.1.0, the version is important as an essential library we use doesn't work in newer versions. We hope to fix this soon.
- Additional R packages:
  a. gd-2.0.33.tar.gz
  b. GDD_0.1-4.tar.gz
- Bioconductor package geneplotter_1.5.4.tar.gz installed in R.
- MySQL installed (should work with all version supporting InnoDb table engine, tested with 4.0.* versions).

Note: CoCo uses Hibernate (http://www.hibernate.org/) for database access and management. You should then be able to easily deploy CoCo on other RDBMS.

Note about Tomcat JVM Memory: CoCo requires quite some memory in specific situations. This might lead to OutOfMemoryError. To prevent this, edit the startup.sh script (in <TOMCAT_HOME>/bin) and add the line just before last line:

```
export CATALINA_OPTS=-Xmx750m
```

2. Installation Overview

For the impatient, here is the complete list of instructions to get CoCo quickly installed and configured

Important: Tomcat, MySQL and R (including required packages) must already be already installed

<u>Installing R libraries (you certainly need to be root)</u>:

- untar, conf, make, make install for gd-2.0.33.tar.gz
- R CMD INSTALL GDD_0.1-4.tar.gz
- Use Bioconductor installation procedure to install geneplotter/biobase/annotate package. You can also go for the easy solution and install the whole Bioconductor. Please see instructions at http://www.bioconductor.org/docs/install-howto.html

<u>Installation steps</u>

1. download coco.tar.gz and put it in a freshly made e.g. 'coco' directory.
2. `tar -xzvf coco.tar.gz` => creates a "`coco`" directory that we'll refer to in the rest of this document as `<COCO_HOME>.`
3. Create coco db in MySQL : log in as root and do:
   - `CREATE DATABASE coco;`
   - `use coco;`
   - `\. <COCO_HOME>/src/sql/coco.sql`
   - `GRANT ALL ON coco.* TO cocouser@<servername> IDENTIFIED BY '<a_password>';`
4. `cd <COCO_HOME>/ant`
5. edit `build_ant.properties` and position required properties (especially adapt db pwd to use for `cocouser` and `db.url` to reflect the MySQL server name!)
6. Manually create the directory that you specified in the property `coco.data.home` in `build_ant.properties file` and make sure that the user running tomcat has full access rights on it (rxw)
7. `ant configure`
8. Install R libs in a shell by typing (you might have to log in as a user having permissions to remove/install R libs):
   - `gunzip, untar, configure, make, make install for gd-2.0.33.tar.gz` (if not already done)
   - `R CMD INSTALL ../lib/R/GDD_0.1-4.tar.gz` (if not already done)
   - `R CMD REMOVE gff3Plotter (!! only if not the first installation !!)`
   - `R CMD INSTALL ../lib/R/gff3Plotter_<version>.tar.gz`

9. In a shell type (you should still be in the `<COCO_HOME>/ant` directory) :
   - `ant install-example`
   - `ant deploy`
   - `chmod 755 <TOMCAT_HOME>/webapps/coco/WEB-INF/lib/*`
   - `ant update-genomes (might take few minutes)`
10. Point your browser to http://localhost(:tomcat_port)/coco and login as admin/admin

Should you fail at any point, re-start from step 7 (or before) and ignore step 8 if it was successful.

3. Detailed Installation Steps

This section explains how to install CoCo step by step.

3.1 Download CoCo

Download the `coco.tar.gz` archive (e.g. from furlonglab.embl.de) and put it in a freshly made e.g. 'coco' directory.

3.2 Uncompress the archive

In a shell, uncompress `coco.tar.gz` e.g.:

```
> tar -xzvf coco.tar.gz
```

This creates a "`coco`" directory that we'll refer to in the rest of this document as `<COCO_HOME>`.
`<COCO_HOME>` contains following directories:

- "`src`" : where all sources are stored (JAVA, Perl, R, SQL)
- "`ant`" : contains ant build file (and build_ant.properties) that allows you to perform a wide range of tasks from building to managing CoCo
- "`doc`" : documentation material
- "`web`" : contains JSPs and web application definition XML files
- "`conf`" : contains CoCo property files
- "`demo`" : some demo data files
- "`logs`" : empty dir used to create log files
- "`lib`" : all needed JAVA and R libraries
- "`template`" : configuration file templates used by the ant (re)configure task to generate installation specific property files

3.3 Create the CoCo SQL Database

Create a '`coco`' database in MySQL (or another RDBMS) and a '`cocouser`' with all rights on '`coco`' database.

Note: CoCo uses Hibernate (http://www.hibernate.org/) for database access and management. You should then be able to easily deploy CoCo on other RDBMS.
Note 2: To date, CoCo has been used on MySQL 4 only but nothing should speak against using any InnoDb MySQL version.

Practically:
- create a database 'coco' in mysql and a 'cocouser' with all rights on this 'coco' db
Go to `<COCO_HOME>/src/sql` and execute the `coco.sql` to create tables in coco db

In MySQL these tasks can be performed using following commands:

```
> mysql –u root –p<ROOT_PWD>

you should now be logged in mysql as root or any user with sufficient
privileges, then execute:

         ➢ CREATE DATABASE coco;
         ➢ use coco;
         ➢ \. <COCO_HOME>/src/sql/coco.sql
         ➢ GRANT ALL ON coco.* TO cocouser@<servername> IDENTIFIED BY
           'coco';
         ➢ exit;
```

Note: if your MySQL doesn t have any server name, simply use localhost

Make sure your settings work by trying to login as cocouser using the mysql console tool, and use coco database i.e.:

```
         ➢ mysql –u cocouser –pcoco –h <servername>
         ➢ use coco;
         ➢ show tables;
         ➢ select count(*) from genes;
```

If login succeed, you can go on…

3.4 Set up project

To be able to configure and build CoCo, you need to give Ant a few properties:

- Go `<COCO_HOME>/ant`
- Edit build_ant.properties and provide required properties (help embedded)
- Manually create the directory that you specified in the property `coco.data.home` in `build_ant.properties file` and make sure that the user running tomcat has full access rights on it (rxw)
- Then run:
  ```
  > ant configure
  ```

3.5 Install required R library

CoCo needs different R library to be installed. In addition CoCo uses functions from Bioconductor packages. To ease things, simply install the whole Bioconductor following the procedure explained at http://www.bioconductor.org/docs/install-howto.html.

Alternatively, make sure to have the following packages (from Bioconductor):

- Geneplotter
- Annotate
- Biobase

To install R libraries that come with CoCo, please read following instructions.

Note: you might have to log in as a user having permissions to remove/install R libs

If not the first installation:

- `R CMD REMOVE gff3Plotter`

Then:

- `gunzip, untar, configure, make, make install for gd-2.0.33.tar.gz`
- `R CMD INSTALL ../lib/R/GDD_0.1-4.tar.gz`
- `R CMD INSTALL ../lib/R/gff3Plotter_<version>.tar.gz`

3.6 Update Hibernate Configuration

Important: This step is **optional** if you use MySQL as the `hibernate.cfg.xml` generated by the `ant configure` task (in "Set up project") generated this file for you already. You can check it out. If you change database properties in the future, this is anyway how you can tell hibernate about the changes.

Procedure:
Edit the Hibernate Configuration file "`hibernate.cfg.xml`" located in `<COCO_HOME>/src/java`.
Update the three following lines with MySQL '`coco`' database information:

```
<property
name="hibernate.connection.url">jdbc:mysql://localhost:3306/coco
</property>
<property name="hibernate.connection.username">cocouser</property>
<property name="hibernate.connection.password">aPWD</property>
```

Replace values with appropriate settings. Note that if you use another RDBMS than MySQL, you'll certainly have to update these additional properties:

```
<property
name="hibernate.connection.driver_class">com.mysql.jdbc.Driver</property>
<!-- dialect for MySQL -->
<property name="dialect">net.sf.hibernate.dialect.MySQLDialect
</property>
<property name="hibernate.show_sql">false</property>
<property name="hibernate.use_outer_join">true</property>
```

3.7. Configuring CoCo

CoCo is configured and maintained using a simple property file named coco.properties and located in <COCO_HOME>/conf. This file has been generated when you ran the ant configure task (in "Set up project"). The file generated is a minimum file that needs to be filled with correct values reflecting your organisms / chips.

If this is the first time you install CoCo, we recommend you to jump directly to "Installing demo data" and test your installation before configuring CoCo with your own data.
Alternatively please refer to "CoCo Management and Maintenance" to learn how to fill in properties. The coco.properties file contains help describing properties and explaining how you should add new ones.

3.8 Installing demo data

CoCo comes with a set of example files that let you run CoCo with further configuration and then check that CoCo is fully functional before configuring your final CoCo server.

Example files contains all files necessary to work with the Drosophila melanogaster genome (genome annotation release 4.0). In addition, a user.properties file (needed for login) is available and can be used later as a guide to add new users. To install demo data, simply run the ant task "install-example", i.e.:

```
➢ cd <COCO_HOME>/ant
➢ ant install-example
```

Note: the coco.properties file in <COCO_HOME>/conf has now been replaced with one containing properties to use demo data. We suggest you to use this file as a starting point when adding your own data in latter steps.

Once done you can login using the username/pwd combination "admin"/"admin" and try to create your first configuration using demo data available in <COCO_HOME>/demo/chip-chip and <COCO_HOME>/demo/expro. These directories contain ChIP-chip results obtained for Drosophila melanogaster Mef2 transcription factor and time series gene expression data using an over-expression of Mef2, respectively. These data are a subset of published results (Sandmann et al. Dev Cell, June; 10(6): 797-807)

3.9. Build the coco.war and deploying CoCo

```
➢ cd <COCO_HOME>/ant
➢ ant buildwar
```

OR (if you deploy CoCo locally):

```
➢ ant deploy
```

A file named coco.war should now be present in <COCO_HOME>/dist. If you haven't executed "ant deploy", you can copy this file in <TOMCAT_HOME>/webapps/ dir, wait a bit to let Tomcat the time to deploy coco and point your browser to http://localhost/coco (assuming tomcat listens the port 80) and you should see the CoCo login page.

Once Tomcat deployed CoCo, execute:

```
➢ chmod 755 <TOMCAT_HOME>/webapps/coco/WEB-INF/lib/*
```

Important: Before logging in, you must create a user.properties file and place it in the directory you indicated in `build_ant.properties` file, property `coco.data.home`. This has been done for you in you choose to install demo data.

3.10 CoCo Management and Maintenance

Common installation (and management) tasks are:

- Define Genome Annotation(s)
- Define Tiling array(s)
- Define LinkOut
- Define InSitu default file(s)
- User list

The first tasks are realized by adding information in the `coco.properties` while adding users in done in `user.properties`.

Important considerations about coco.properties

The `<COCO_HOME>/conf/coco.properties` file has been generated when you executed “`ant configure`”. Running “`ant configure`” again will certainly replace this `<COCO_HOME>/conf/coco.properties`. To get a `coco.properties` from scratch, use “`ant reconfigure`”.

Hence, you might want to reflect the changes you make in `<COCO_HOME>/conf/coco.properties` in `<COCO_HOME>/template/coco.properties` or edit `<COCO_HOME>/template/coco.properties` directly and launch ”`ant reconfigure`” after modifications.

Summary:

1. When editing coco.properties, you always have the choice between:
   - Editing `coco.properties` in `<COCO_HOME>/template/` followed by “`ant reconfigure`”=> recommended at installation time

   - Editing `coco.properties` directly on the server (the file is located in (`<TOMCAT_HOME>/webapp/coco/WEB-INF/classes/org/embl/coco/ )` and manually reflecting changes locally => recommended once your server is already in production (i.e. you don t want to restart Tomcat)

2. After modifying `coco.properties` do one of the following:

- At installation time, after edition of `coco.properties in COCO_HOME/template/`, do:
  - ➢ `cd COCO_HOME/ant`
  - ➢ `ant (re)configure`
  - ➢ `ant uploadgenomes-all`
  - ➢ `ant deploy`

- At maintenance time, after edition of `coco.properties` on the server, do:
  - ➢ `login as admin`
  - ➢ `go to the "Admin Tools" menu (only available when logged in with the admin role)`
  - ➢ `Click on the "Reload application properties" link.`
  - ➢ `Done!`

7.1 Defining new genomes (in coco.properties)

Defining new genomes in CoCo is certainly the most tedious task in CoCo (but still very easy!). For this you need to:

1. Add the taxid (if not yet listed) of your organism in the `genome.supported.taxids` property (this property accepts a comma-separated list of NCBI taxids)
2. Add the genome annotation version of the genome annotations in the corresponding `supported.genome.versions.<taxid>` (this taxid-specific property accepts a comma-separated list of genome annotation versions)
3. List (if not done already) the chromosome names of this organism in the taxid-specific `chrname.list.<taxid>` (this taxid-specific property accepts a comma-separated list of chromosome names). Note that the order in which you list the chromosomes are kept in CoCo display.
4. For each chromosome, define a `genome.layout.gff.<taxid>.<version>.<chrName>` which value in the absolute path to the annotation file in GFFv3. For a complete description of this format, please see http://flybase.bio.indiana.edu/annot/gff3.html or http://www.sanger.ac.uk/Software/formats/GFF/GFF_Spec.shtml

Note: gene symbols, names and synonyms are extracted from the ninth column. Used fields (case-sensitive) are "ID" (ends up as gene symbol), "Name" (ends up as gene name), "Dbxref", "dbxref_2nd" and "synonym" (all end up as synonyms). For an example, look at the demo data shipped with CoCo.

Note 2: to speed up computation time, we encourage you to filter GFF you download and keep only relevant annotation types (take a look at `gff.genome.feature.types` to know which types are relevant)

5. Add `genome.layout.rdata.<taxid>.<version>.<chrName>` properties the same way you positioned GFF paths at step 4

Note: These Rdata files don't yet exist. You'll generate them at next step in which these `genome.layout.rdata.<taxid>.<version>.<chrName>` will be used.

6. Run `ant update-genomes`

7.2 Defining new Tiling Arrays (in coco.properties)

1. Add the chip name (used for display) in `supported.chipnames` property (this property accepts a comma-separated list of chip names)
2. Add a feature map (or clone map) property by setting a new `tiling.chip.clone.map.<chipname>` which value is the absolute path to the features genomic position file. This file is a 4-columns tab-delimited file holding the feature_id, the chr, the start and stop position of the chip feature. Note that ChIP-chip result files must use these ids. Take a look at demo data for an example.
3. Add a `genome.<chipname>` and `genome.version.<chipname>` property for the microarray which values must refer to taxid and genome version present in the configuration file.

7.3 Defining LinkOut (in coco.properties)

LinkOut allows you to enrich CoCo interface by defining links to other web resources. *LinkOut* are build using gene symbol values. Just take a look at demo data to see how to define a *LinkOut*

Note: Defining *LinkOut* is optional.

7.4 Defining InSitu default file(s) (in coco.properties)

To our experience, preparing in situ files might reveal to be quite time-consuming. Hence CoCo let you define default in-situ result files (one per organism) that will be available to all users.

To define such a default file, simply add a `insitu.default.file.<taxid>` property. The value should be the absolute path to a tab-delimited file holding in-situ results.

Format is a simple as:
Column 1: GeneSymbol
Column 2: dev_stage
Column 3: anatomy

You certainly wonder what terms to use in columns 2 and 3? Well, it is up to you! The most important is to let your users know what terms have been used so that they can build relevant term lists to give to CoCo!

Note: Defining default in-situ result files is optional.

7.5 Defining User(s) (in user.properties)

Well simply add a line holding required description for the new user(s). You'll find examples and format description in the demo `user.properties` file. Users can belong to different groups and have multiple roles. In such situation, list all roles/groups using a comma separated value list in the appropriate column.

Note about groups: guest and temporary users might have no group, in this situation simply register them in the special "nogroup" group. But be aware that all "nogroup" users might see each other data.

Roles known in CoCo:

- "user" : the role for every user
- "admin" : an admin sees everything, only admin have access to the "Admin Tools " menu.
- "groupLeader" : a groupleader will see all data of his group even when not shared with the group.